APPLICATION

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TITLE: CELLULAR PHYSIOLOGY WORKSTATION FOR AUTOMATED DATA ACQUISITION AND PERFUSION CONTROL

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CELLULAR PHYSIOLOGY WORKSTATIONS FOR AUTOMATED DATA ACQUISITION AND PERFUSION CONTROL

Government Support

Microfiche Appendix

One microfiche appendix is filed with this application. Microfiche appendix A contains a total of 1 microfiche and 18 frames.

Background of the Invention

1.   Field of the Invention

The present invention relates to apparatuses for data acquisition and perfusion control in the analysis of cellular physiology and electrophysiology and to methods for automated perfusion and membrane voltage and current measurement for physiological and electrophysiological analysis.

2.   Description of the Background

Cell membranes communicate information from the extracellular environment by means of receptor and channel proteins located within the cell membrane. Receptor proteins are gated by molecules which can bind to the receptor and signal that a binding event has taken place, often by triggering the opening of ion channels through which ions such as sodium and chloride ions can flow. Ionic flux across a cell membrane generates electrical current that can be measured with appropriate recording equipment. Electrophysiological analysis is widely used today to study the pharmacology and biophysics of membrane proteins.

An expression system utilizing unfertilized eggs, or oocytes, taken from the South African clawed frog, *Xenopus laevis*, is a preferred material for electrophysiological

studies of receptor and ion channel function. *Xenopus* oocytes have the ability to synthesize functional proteins when microinjected with exogenous mRNA or cDNA constructs.

In electrophysiological analysis, an oocyte is
5   electrically connected to intracellular voltage and current measuring and clamping devices. Detection of an electrophysiological response may comprise steps of applying appropriate receptor ligands and adjusting the holding potential manually and measuring any changes in membrane
10   voltage or current.

Recently, electrophysiological analysis of *Xenopus* oocytes has been actively applied to many fields. In particular, electrophysiological analysis has been used for the study of membrane protein function, such as the function
15   and pharmacology of membrane receptors, voltage-gated ion channels, molecular transporters and ion pumps. Defined combinations of recombinant subunits, chimeric proteins, or mutagenized constructs can be efficiently reconstituted in the oocyte membrane for electrophysiological analysis. For
20   such analysis, the oocyte response may be monitored using intracellular recording, patch clamp and internal perfusion techniques.

It has been difficult to achieve a highly reproducible and reliable assay or to achieve quantitative analysis of
25   electrophysiological response by conventional manual perfusion and membrane potential measurement techniques. These techniques have many shortcomings because of variabilities due to human errors, operator fatigue and inconsistencies between operators, and less than optimal
30   reproducibility and reliability. Further, the perfusion and detection steps typically require long and complicated manual manipulations which create additional problems. The cultured cell becomes less viable with time and it is difficult to control the temperature and oxygen tension.
35   The limited dexterity of even the most experienced operator

limits the number of experiments may be performed on one cell. Reliance on human operators has resulted in reaction times that are considerably longer than theoretically possible.

5 Conventional systems for analysis of cells have attempted to address some of the problems of automated cell analysis. These systems have suffered generally from inability to individually measure a physiological response of a cell. Examples of systems that do not address
10 individual physiological measurements include Kearney, Engström, Fränzl et al. and Capco et al.

Kearney (US patent no. 5,424,209), discloses a system for culturing and testing of cells. This culturing and testing system was designed for the culturing and testing of
15 cell populations and not individual cells.. Engström (US patent no. 5,312,731) discloses a method and apparatus for studying a reaction pattern of a cell or cell aggregate during perfusion with different media. The system is limited to analysis of cell response of a through
20 transmission microscopy. Fränzl et al., (US patent no. 5,432,086) discloses an apparatus for the automatic monitoring of microorganism culture. The system is limited to the monitoring of microorganism growth and multiplication by an impedance measuring process. Capco et al., (US patent
25 no. 4,983,527) discloses a method for detection of tumor promoting compounds. Amphibian oocytes are contacted to a tumor promoting compound and the oocytes are examined visually to detect a change in the size of the light/dark hemisphere of the oocyte. Capco's disclosed method is
30 limited to contacting the oocytes to one solution comprising a candidate tumor promoting compound.

Summary of the Invention

The present invention overcomes the problems and disadvantages associated with current strategies and designs

and provides novel apparatus and methods for the study of membrane physiology.

One embodiment of the invention is directed to cellular physiology workstations that enable automated execution of

5 experimental protocols for electrophysiological experiments and for the development of more complex protocols based on extended recording sessions. As currently developed for oocyte electrophysiology, the apparatus comprises one or more custom-built recording chambers, a perfusion control

10 system designed for rapid application of about 2 to about 16 or more solutions under automated control, software-based virtual instrumentation developed to automate the execution of experimental protocols, and a data acquisition and control platform which integrates the entire system. The

15 system is fully customizable through a sophisticated object-oriented programming language and can be easily adapted to applications such as patch clamp electrophysiology, calcium imaging studies, confocal microscopy and other applications where perfusion control and data acquisition need to be

20 tightly integrated.

Another embodiment of the invention is directed to apparatus for reproducibly detecting the electrical response of a cell to an agent. The apparatus comprises a plurality of recording chambers. Each chamber is designed to contain

25 one or more cells such as, for example, one or more *Xenopus* oocytes. Means are provided to perfuse each recording chamber with a plurality of perfusion solutions. Each perfusion solution may contain a different concentration of one or more agent. A plurality of electrodes such as, for

30 example, a voltage measuring electrode, a current injecting electrode or a glass patch electrode, may be connected to each cell to measure the electrical response of the cell to the presence, absence or change in concentration of the agent. The electrical response may also be measured at

35 various holding potentials.

Another embodiment of the invention is directed to automated apparatuses for electrophysiological measurement which comprises injecting means, such as a needle, for delivering an injection solution into the cell. The

5   injection solution may comprise a second agent, a protein, a nucleic acid or a combination thereof. The nucleic acid may be, for example, DNA, RNA or PNA. PNAs, peptide nucleic acids or protein nucleic acids, are synthetic polymers capable of hybridizing in a sequence specific manner with

10 natural nucleic acids.

Another embodiment of the invention is directed to methods for reproducibly detecting a physiological response of a cell to a agent. A cell such as, for example, a *Xenopus* oocyte, is perfused using an automated perfusion

15 system with a plurality of solutions, which may comprise different concentrations of one or more agents, and the electrophysiological response of the cell measured. The automated perfusion control system may be, for example, a gravity fed flow through perfusion system. The automated

20 perfusion control system may have an optimized lag time of less than about 100 milliseconds and a rise time of less than about 140 milliseconds such as less than about 70 milliseconds.

Another embodiment of the invention is directed to

25 assays for detecting a substance which affects cellular physiology. A cell is injected with a nucleic acid such as, for example, DNA or RNA encoding a membrane receptor. The cell is perfused with a plurality of solutions comprising different concentration of said substance using an automated

30 perfusion system. A change in cellular electrophysiology of the cell is detected to determine the effect of the substance. The period of time between the injecting step and the perfusing and measuring steps may be between about one hour to about 15 days.

Another embodiment of the invention is directed to a
substance detected by the assay. A candidate substance is
used and the assay is performed to detect a desirable
effect. A substance capable of inducing a desirable effect
5   is identified by the assay.

Another embodiment of the invention is directed to a
kit for performing the assay. The kit may comprise reagents
and biosensors for the performance of the assay.

Other embodiments and advantages of the invention are
10   set forth, in part, in the description which follows and, in
part, will be obvious from this description and may be
learned from practice of the invention.

Brief Description of the Drawings

Figure 1   depicts an automated oocyte perfusion control
15   system according to one embodiment of the present
            invention.

Figure 2   depicts a voltage clamp and recording chamber
            connection according to one embodiment of the
            present invention.

20   Figure 3   depicts an internal perfusion control system
            according to one embodiment of the present
            invention.

Figure 4   depicts a block diagram showing information
            transfer between the system components.

25   Figure 5   depicts components of the perfusion system.

Figure 6   depicts an instrumentation graphical user
            interface according to one embodiment of the
            present invention.

Figure 7   depicts responses for several ligand-gated ion
30   channels expressed after injection of the oocytes
            with rat brain mRNA.

Figure 8   depicts averaged kainate dose-response data from
            four oocytes injected with GluR6 cDNA.

Figure 9   depicts the effect of pregnenolone sulfate on
            kainate dose-response curve in oocytes injected
            with rat brain poly A$^+$ mRNA.

Figure 10 depicts the measurement of pregnenolone sulfate
5   IC50 in oocytes injected with rat brain poly A$^+$
            mRNA.

Figure 11 depicts the effects of steroids on recombinant
            GluR6 kainate receptors.

Figure 12 depicts steroid IC50 determinations for
10   recombinant GluR6 receptors.

Figure 13 depicts the kainate concentration dependence of
            pregnenolone sulfate inhibition.

Figure 14 depicts inhibition of kainate responses by
            stimulation of metabotropic glutamate receptors.

15 Figure 15 depicts γ-aminobutyric acid (GABA) dose-response
            curves revealing reproducibility of GABA EC_{50}.

Figure 16 depicts a high-resolution dose-response curve
            generated by the cellular physiology workstation.

Figure 17 depicts current responses to 30 consecutive
20   applications of 100 μM GABA.

Figure 18 depicts determination of reversal potential
            generated automatically by the cellular physiology
            workstation.

Figure 19 depicts an examination of the endogenous calcium-
25   dependent chloride current ($I_{Cl^-(Ca)}$) present in
            native oocytes.

Figure 20 depicts an alternate embodiment of the perfusion
            control system of the present invention.

Figure 21 depicts a font panel which aids in the creation
30   and editing of recipe files.

Detailed Description of the Preferred Embodiments

    Performing multiple electrophysiological measurement on
a plurality of cells while maintaining consistency between
individual experiments is problematic.   Difficulties

associated with reproducibility and reliability render any
more than two or three measurements suspect. The present
invention overcomes these problems using automated perfusion
systems and methods that are capable of controlled and

5   consistent perfusions on a plurality of recording chambers.

        The cellular physiology workstation of the invention,
when applied to electrophysiology, has several advantages.
Automation allows experimenters to maximize the amount of
data that can be obtained from a given cell during the

10   limited viability of microelectrode-impaled cells.
Automation also increases the speed and throughput of
electrophysiological experiments while reducing errors
associated with manual manipulations and waveform analysis.
Manipulations required during a recording trial such as,

15   toggling switches, applying drugs, measuring the response
and timing the experiment are minimized or eliminated.
Automation also enables the development of more complex
protocols based on repetitive recordings that permit
averaging of data before and after a given manipulation.

20   Finally, automation of experimental protocols makes it
feasible to utilize technician-level operators for the
collection of electrophysiological data. This is
advantageous for drug screening and receptor
characterization. By minimizing timing, perfusion and

25   instrument control manipulations, automation reduces
experimenter fatigue during extended recording sessions.
Protocols which are too complex, fast, or repetitive for
manual performance may be preprogrammed and executed
consistently and reliably with a single keypress. Further,

30   the experimenter may focus on the hypothesis being tested
rather than the mechanics of electrophysiological technique.
Automation enables the precise timing of agent application,
such as drug application, and improves the quality of
experimental data by reducing inadvertent errors,

35   idiosyncratic variations in protocol between different

investigators, and introduction of noise through manual manipulations. Automated waveform analysis reduces measurement errors as well as the post-processing time necessary for the analysis of experimental data, while

5 enabling real-time evaluation of results. Additionally, the efficiency and speed of data generation are increased, thereby allowing mass, parallel screenings of large chemical libraries. This added throughput also allows the researcher the opportunity to test secondary hypotheses that might

10 otherwise have been neglected due to recording time limitations or the tedious nature of the task. The physiology workstation is especially useful and advantageous in pharmaceutical, chemical, and biotechnical research and development. The methods and apparatus of the invention is

15 especially suited for repetitive or complex protocols, such as a drug dose response analysis.

Accordingly, the present invention provides an application-specific integrated workstation, particularly one for cellular electrophysiology such as oocyte

20 electrophysiology, which results in a tremendous reduction in time expenditure in assembly. Additionally, the invention provides an automated workstation which provides greater efficiency and higher productivity. The workstation provides a tightly integrated system comprising recording

25 chambers, perfusion system, data acquisition platform and instrumentation software that enables immediate experimentation without additional set up. At the same time, system flexibility is preserved by allowing selection of amplifiers, microscopes, micromanipulators and other such

30 devices that are most appropriate for the experimenter's requirements.

The apparatuses and methods of the present invention may also be used for techniques such as, internal perfusion of oocytes, patch clamp electrophysiology, brain slice

35 recording, receptor-ligand interactions on cell surfaces,

calcium imaging studies, confocal microscopy, and *in vivo* microdialysis, for example. The system of the present invention may also be used to examine the function of ligand-gated ion channels, voltage-gated ion channels, G-

5   protein coupled receptors, activities across the synapse, molecular transporters, cell-cell interactions and ion pumps. The system may also be useful for screening compound libraries to search for novel classes of compounds, screening members of a given class of compounds for effects

10 on specific receptors, detailed pharmacological characterizations of compounds having receptor effects, rapid evaluation of EC_{50} (potency) and E_{max} (efficiency), investigation of interactions between receptors and rapid characterization of a series of receptor mutants. The

15 invention provides repetitive application, dose-response data generation, evaluation of receptors expressed from poly A^+ mRNA, and evaluation of recombinant receptors such as, for example, γ-aminobutyric acid (GABA) receptors, kainate receptors, and N-Methyl-D-aspartic acid (NMDA) receptors.

20   Examples of agents that may be used for the apparatus and methods of the invention include drugs, receptor agonists, receptor antagonists, neurotransmitter, neurotransmitter analogues, enzyme inhibitors, ion channel modulators, G-protein coupled receptor modulators, transport

25 inhibitors, hormones, peptides, toxins, antibodies, pharmaceutical agents, chemicals and combinations of these agents. Specific agents which may be used for the apparatus and methods of the invention include purinergics, cholinergics, serotonergics, dopaminergics, anesthetics,

30 benzodiazepines, barbiturates, steroids, alcohols, metal cations, cannabinoids, cholecystokinins, cytokines, excitatory amino acids, GABAergics, gangliosides, histaminergics, melatonins, neuropeptides, neurotoxins, endothelins, NO compounds, opioids, sigma receptor ligands,

35 somatostatins, tachykinins, angiotensins, bombesins,

bradykinins, prostaglandins and combinations of these agents.

Another embodiment of the invention is directed to an automated workstation for data acquisition and perfusion
5   control to facilitate electrophysiological measurement. A preferred apparatus and method are described in relation to *Xenopus* oocytes as follows, but it is clear to one of ordinary skill in the art that the described apparatus and methods are broadly applicable to many cell types.

10   Automated Perfusion Control System
        One embodiment of the present invention, depicted in Figure 1, is an automated perfusion control system for electrophysiological studies of *Xenopus* oocytes. The system comprises computer 10, operating instrumentation software,
15   recording chamber 12 designed to receive a cell such as for example, oocyte 32, and perfusion control system 14. Perfusion control system 14, depicted in Figure 1 and Figure 5, comprises valve controller 16, connected to and under the control of computer 10. Valve controller 16 is connected by
20   a plurality of tubes 44 to a plurality of constant flow reservoirs 18 to multi-barrel manifold 20. Each reservoir comprises ventilation means 46 which allow gravity flow of solution from the reservoir 18, through tube 44 when valve controller 16 is open. Valve controller 16 comprises a
25   plurality of solenoid valves. The system may also comprise voltage clamp amplifier 22 connected to and under control of computer 10 via BNC box 24. Voltage clamp amplifier 22 is connected to oscilloscope 26 and to two impaling electrodes 28 and 30 in recording chamber 12.
30   According to one embodiment of the present invention, the automated perfusion control system of the cellular physiology workstation may comprise a plurality of reservoirs 18 containing one or more different perfusion solutions. The reA valve 16 may be used to control delivery

of the fluid to the one or more recording chambers **12**. The fluid valve may be manual or machine operated. A machine operated valve may be controlled directly by computer means within the cellular physiology workstation. The automated
5   perfusion control system may comprise between 2 to about 100 reservoirs, preferably between about 4 and about 20 reservoirs. The automated perfusion control system may optionally comprise a mixing means, such as a mixing chamber, between the fluid valve and the recording chamber.
10   Turning to Fig. 20, an alternate embodiment of the present invention is shown as perfusion control system **14'**. This system **14'** is in most respects substantially identical to perfusion control system **14**, while including a plurality of recording chambers **12** and a plurality of manifolds **20**.

15   Oocyte Preparation
         In a preferred embodiment cells monitored by the cellular physiology workstation are *Xenopus* oocytes. Numerous methods for preparing oocytes and poly A$^+$ RNA are known to those of skill in the art. One method is described
20   as follows.
         Donor animals, female oocyte positive *Xenopus laevis* frogs, are anesthetized in a solution of about 0.15 % Tricaine for about 30 minutes. Ovarian sections are removed through a lateral abdominal incision, after which the
25   incision is sutured with about 4 to about 5 stitches and the frog is allowed to recover in isolation for about 3 to about 4 hours. Ovarian lobules containing the follicular oocytes are immediately rinsed with calcium-free ND96 solution (96 mM NaCl, 1 mM $MgCl_2$, 2 mM KCl, 50 mM Hepes, 2.5 mM pyruvate)
30   and cut into clumps of about 10 to about 20 oocytes. Following 2 mg/ml collagenase treatment (Sigma) at room temperature for about 2 hours, individual oocytes are obtained free of their follicular layer. Selected oocytes (Dumont stage V and VI) are then transferred to 60 x 15 mm

glass petri dishes containing ND96 (96 mM NaCl, 1 mM $MgCl_2$, 2 mM KCl, 50 mM Hepes, 2.5 mM pyruvate) and maintained in an incubator at a temperature of about 18°C to about 19°C.

Poly A^+ mRNA are extracted from brain tissue and neuronal cell culture using a magnetic separation protocol based on the Dynabeads Oligo (dT)25 kit (Dynal, Olso, Norway). Briefly, the protocol utilized magnetic beads that have an attached poly-T moiety which can bind poly A^+ mRNA for separation. Tissue is homogenized, cells are disrupted, and the lysate is added to an aliquot of Dynabeads. Magnetic separation efficiently yields mRNA that is suitable for direct injection into oocytes. Batches of about 20 to about 30 select oocytes are injected with about 50 to about 100 ng of neuronal poly A^+ mRNA. Alternatively, oocytes may be injected with about 30 μl to about 80 μl of RNA prepared from *in vitro* transcription of cDNA clones. In either case, injection may be performed using a Drummond electronic microinjector. Oocytes are then maintained at about 18°C to about 19°C for about 2 to about 4 days to allow protein expression prior to electrophysiological recordings. After incubation, electrophysiological analysis of expressed receptors and ion channels may be performed using a system according to the present invention.

Recording Chamber

A novel intracellular recording chamber for *Xenopus* oocyte electrophysiology has been developed to enable rapid agent application and automated control over perfusion protocols. Recording chamber **12** features a flow-through design in which gravity-feed eliminates the need for pressurization of solution containers and drop-wise removal of perfusate eliminates the need for a vacuum line. As depicted in Figures 1 and Figure 2, oocyte **32** sits in V-shaped groove **34** in fluid chamber **36** with a capacity of about 100 μl and is stabilized against solution flow by

microelectrode impalement. The recording chamber may be adapted to accept more than one cell such as an oocyte. For example, the recording chamber may be adapted to receive about 3, about 10, about 15, or about 100 cells.

5   Optionally, if it is desired to provide more stability against solution flow, additional devices such as needles or electrodes may be employed. This has resulted in rise times (5%-95%) of about 70 msec to about 140 msec for 100 μM GABA responses from oocytes expressing $GABA_A$ receptors, which

10   represents the fastest rise times reported to date for *Xenopus* oocyte electrophysiology. The chamber is preferably made of non-conductive plastic and is clamped onto a microscope stage for stability. In one embodiment the entire chamber is plastic and electrodes **28** and **30** impale

15   oocyte **32**. The physiology workstation may comprise more than one recording chamber such as about 3, about 10, about 15, or about 100 recording chambers.

Electrodes **28** and **30** are connected to operational amplifiers **A2** and **A1** respectively of a voltage clamp

20   amplifier. Operational amplifier A1 measures the voltage difference between the voltage recording electrode **30** and reference electrode **38** which is disposed in KCl well **40**. KCl well **40** preferably comprises about 3M KCl and is in fluid communication with fluid chamber **36** by way of agar

25   bridge **42**. Operational amplifier **A1** feeds the difference between electrode **30** and electrode **38** to operational amplifier **A2**. Operational amplifier **A2** compares the voltage difference with the desired voltage difference, Vcom, and outputs current to injecting electrode **28** to maintain the

30   oocyte membrane at a desired potential. In a preferred embodiment, the desired potential may be any value between about 200 mV to about -200 mV such as about -60 mV and is stepped up to any value between about 200 mV to about -200 mV such as about -100 mV during agent application to

35   increase ionic driving forces.

The electrical response detected by the cellular physiology workstation may be a membrane potential or a membrane current. After detection the electrical response may be recorded by a recording means such as, for example, a
5 digital recorder, a computer, volatile memory, involatile memory, a chart recorder or a combination of recording devices. The apparatus may further comprise means for controlling the temperature and oxygen level of the recording chamber.
10 Fluid chamber **36** has a port to which multi-barrel manifold **20** may be attached for delivery of solutions (Figure 1). Perfusate drips through an aperture into a plastic perfusate collection chamber which feeds into a disposal bottle. Flow rates of about 1.5 ml to about 3.0 ml
15 per minute can be achieved with adjustment of the height of the reservoir bank. Higher flow rates can be obtained with pressurization but are subjected only to the limitation of the stability and integrity of microelectrode impalement of the oocyte membrane. Minimum lag time and onset times are
20 important for high sensitivity experiments. One advantage of the cellular physiology workstation is a recording chamber which minimizes dead volume and lag time. While pressurization and vacuum may be used, it is not required. The physiological workstation is capable of optimized lag
25 times (valve switching to response onset time) of less than about 100 msec, such as less than 50 msec, and onset times (time to go from about 5% to about 95% of maximum amplitude) of between about 70 milliseconds to about 140 milliseconds are obtained with about 100 μM γ-aminobutyric acid (GABA)
30 responses in oocytes injected with chick brain poly(A)$^+$ RNA. The solution exchange time is about one second for 50% exchange and about 8 seconds for 90% exchange as measured by depolarization induced by switching from normal Ringer to high potassium Ringer. An agar bridge may be used to

establish electrical contact with the reference electrode through an attached KCl well.

In one embodiment, a two-electrode voltage clamp is used with two intracellular microelectrodes pulled on a

5   Flaming-Brown micropipette puller (Model P80/PC; Sutter Instrument Co.). These electrodes have input resistances of about 2 mega-ohms to about 4 mega-ohms when filled with a solution comprising about 3M KCl. Microelectrode positioning and impalement of the oocyte may be performed

10   under micromanipulator control.

The recording chamber may further comprise means for controlling gas levels such as oxygen, nitrogen, and carbon dioxide levels. In addition the recording chamber may further comprise means for temperature control.

15   Configuration of the recording apparatus

The recording chamber herein described is designed to be clamped directly onto the microscope stage. The small size and novel design of this chamber permits two such chambers to be clamped side-by-side onto the stage of an

20   unmodified Nikon SMZ-10 microscope for visual monitoring of the cells during experimentation. With slight modification and extension of the microscope stage, up to 5 such recording chambers could be so utilized. The microscope head is mounted on a sliding bracket to facilitate panning

25   of the viewing field across the row of parallel recording chambers. For simultaneous recordings from multiple oocytes, each of these recording chambers could have two independent micromanipulators for positioning of the voltage-recording and current-injecting electrodes. At

30   least about six micromanipulators can be positioned around the microscope stage. Ten or more micromanipulators might be positioned about the recording chamber by the careful positioning and by the use of small footprint micromanipulators.

The recording chamber may optionally comprise means for receiving and automatically positioning a cell within said recording chamber. Positioning means may comprise indentations in said recording chamber for the cells such as

5   oocytes to settle. Other positioning means may also comprise robotic means, and artificial intelligence means for the proper positioning of the cells. Cells may be held in place after positioning by impaling probes which may or may not be a biosensor. The simplest impaling probe for

10 immobilizing a cell may be a glass needle. Other forms of immobilizing cells such as oocyte may comprise, for example, adhesives, vacuum and indentations.

The recording chamber may optionally comprise means for positioning said one or more biosensors to detect a response from the cell. Means for positioning may be in the form of

15 a template with biosensors spaced regularly to proximate cells positioned by the automatic cell positioning means. The biosensors may be, for example, electrodes, patch clamps or microscopes. The positioning of biosensors such as, for

20 example electrodes, may involve the puncture and penetration of the cellular membrane. If the cells are of uniform size, such as *Xenopus* oocytes, the depth of penetration may be preset and fixed. Alternatively, the positioning and the depth of penetration may be determined by an automatic

25 positioning system tailored for the specific cell type. The automatic positioning system may comprise for example, feedback and robotic mechanism which may be computer controlled for determining the proper position and depth of the probes.

30 Injecting means

The physiological workstation may comprise optional means of injecting one or more injection solutions into said oocyte between the culturing and measuring step. The

injection solution may comprise an agent. The agent may be
a chemical, a protein or a nucleic acid.

Examples of agents that may be injection include
proteins, DNA, RNA, PNA, receptor agonists, receptor
5 antagonists, neurotransmitter, neurotransmitter analogues,
enzyme inhibitors, ion channel modulators, G-protein coupled
receptor modulators, transport inhibitors, hormones,
peptides, toxins, antibodies, pharmaceutical agents,
chemicals and combinations of these agents. Specific agents
10 which are of interest include purinergics, cholinergics,
serotonergics, dopaminergics, anesthetics, benzodiazepines,
barbiturates, steroids, alcohols, metal cations,
cannabinoids, cholecystokinins, cytokines, excitatory amino
acids, GABAergics, gangliosides, histaminergics, melatonins,
15 neuropeptides, neurotoxins, endothelins, NO compounds,
opioids, sigma receptor ligands, somatostatins, tachykinins,
angiotensins, bombesins, bradykinins, prostaglandins and
combinations of these agents.

Perfusion Control System
20   Agent solutions are held in a plurality of plastic
reservoirs, **18**, each of which has a capacity of about 15 ml
to about 50 ml. Reservoirs are constructed so as to
maintain a constant flow rate regardless of the level of
solution in each reservoir. A constant flow rate is
25 important to ensure reproducibility of responses since onset
of response is influenced by agent application rate. As
depicted in Figure 1, each reservoir **18** contains glass
siphon **44** extending down through its cap into the solution
and vent line **46** also extending into the solution that
30 equilibrates chamber pressure for maintenance of constant
flow rate. Solution-flow is by gravity feed and the flow
rate can be controlled by adjusting the height of the
reservoirs. Other method of solution flow such as vacuum,
pressure or pumping may also be used. Dropwise removal of

solution through the efflux line creates negative pressure
in the chamber which is equilibrated by means of the vent
line.   In one embodiment, up to 16 different agent solutions
can be prepared and placed in a rack designed to hold 16
5   reservoirs. Additional reservoirs may also be provided
depending on the number of ports in manifold **20**.

Multi-barrel manifold **20** receives input lines from the
reservoirs **18** and provide a point of convergence for the
different solutions. Flexible tubing, such as Tygon™, of
10   about 0.9 mm inner diameter, carries solution from each
solenoid valve of valve controller **16** to a separate barrel
on manifold **20**, where the lines converge to an output port
that can be connected directly to recording chamber **12**.
Other models having additional barrels may also be used.
15   These manifolds are custom-made from glass capillary tubing
fitted with plastic tubing adaptors and have been made in 8-
and 16-barrel models. These designs minimize internal dead
volume so as to enable rapid agent application and minimal
dilution of solutions.
20   The perfusion controller system functions to translate
digital output from an analog/digital input/output (MacADIOS
II) card **60** (Figure 4), which is connected to computer **10**,
into signals which can be utilized to switch relays and
solenoid valves that control perfusion. Solution flow
25   between the constant-flow reservoirs and multi-barrel
manifold is preferably controlled via 16 miniature teflon-
coated solenoid valves (Lee Valve Co.; Essex, CT). These
valves are particularly suitable because of their corrosion
resistance, biocompatibility and power requirements. Other
30   valves exhibiting these characteristics may also be used.
These valves may be actuated by a direct current voltage
supplied from a direct current power source which would
eliminate electrical hum. Direct current power sources may
be, for example, a direct current power supply or a battery.
35   The value of the direct current voltage may be, for example,

12 volts. The valves are interfaced to the instrumentation software through an analog/digital input/output module **16** serving as valve controller **16**. The digital I/O module preferably comprises a 16-channel backplane (OPT022, #PB1

5 6HC) fitted with DC output modules (OPT022, #ODC5) to which the solenoid valves are connected. The digital I/O module connects to the digital out port of the analog/digital input/output (MacADIOS II) card via a flat ribbon cable and has an external 7.5V power supply (Figure 4). The perfusion

10 control system containing the solenoid valve assembly and the digital I/O module, is housed in a box into which flow lines enter from the agent reservoirs and exit to the multi-barrel manifold. This gravity fed perfusion control system incorporates solenoid valves to allow computer controlled

15 switching.

In another embodiment, the perfusion control system selects between five different agent valves using transistor-based circuits in digital I/O module 16 to switch between buffers and agent inputs in response to transistor-

20 transistor logic (TTL) signals sent on two digital lines from a data acquisition card such as a MacADIOS II card. Perfusion automation at this point provides the ability to turn on a buffer valve at the start of data acquisition, switch to a preselected agent valve at an indicated time,

25 then switch back to buffer for agent washout, all under control of computer **10**. This perfusion control system enables timely agent application protocols and removes the necessity of having to manually switch valves at appropriate times, tedious manipulations prone to experimental error and

30 generation of noise.

According to another embodiment of the invention a chamber for internal perfusion of oocytes is depicted in Figure 3. A glass perfusion chamber may be drawn from a Pasteur pipette tip that has been melted down to form a

35 narrow aperture. Increase of fluid level in the chamber

leads to formation of a high resistance seal between the
devitellinized oocyte membrane and glass. Multi-port
manifold **20** feeds into a perfusion line **46** which is threaded
close to the oocyte **32** for agent delivery and thus external
5   perfusion. Perfusate is removed through line **48** which
maintains a constant fluid level by means of a peristaltic
pump **50**. Electrodes **28** and **30** may be in electrical contact
with oocyte **32** by a fluid retention sleeve **52** through which
a perfusion cannula **54** may be advanced for oocyte membrane
10   rupture and internal perfusion. Chloridized silver wire **56**
provides conduction between reference electrode **38** in well
40 and the chamber fluid. The internal perfusion controller
of Figure 3 allows introduction of drugs or enzymes into the
oocyte cytoplasm and control of intracellular composition.
15   The use of this perfusion controller allows control over
external and internal perfusion.

Computer Control and Data Analysis Means

Instrumentation software, currently based on the
SuperScope II v1.43 programming environment (GW Instruments;
20   Somerville, MA), preferably operates on computer **10**.
SuperScope II provides a sophisticated graphical environment
which facilitates the development of virtual instruments
that are used for data acquisition and instrument control.
On-screen representations of buttons, dials and sliders can
25   be programmed to activate desired software routines using an
object-oriented programming language and are used to build
application-specific instruments. Other programming
environments, for example, LabView™ by National Instruments
(Austin, TX) or similar products or a general purpose
30   programming language such as C++ can also be used to achieve
to achieve similar interface. An instrument for oocyte
electrophysiology may integrate agent delivery, instrument
control, data acquisition and waveform analysis through an
on-screen, mouse-driven interface. Figure 6 shows a screen

shot from one embodiment of such an instrument. While the
virtual instrument programs such as SuperScope II or LabView
provides significant convenience for the user,
instrumentation software may also be written by those of
5   skill in the art.

A virtual instrument of oocyte electrophysiology is
depicted in Figure 6. The virtual instrument was created
using the SuperScope II development environment (GW
Instruments, Inc.) to allow complete experimental control
10   through a graphical user-interface. On-screen markers (M1-
M4) can be moved via the mouse to set the duration of the
PREPULSE PHASE (the interval between markers M1 and M2), the
DRUG APPLICATION PHASE (the interval between markers M2 and
M3), and the *WASHOUT PHASE* (the interval between markers M3
15   and M4). For each phase, the perfusion controls **1** are set
to select the valves controlling the wash solutions. The *VC
COMMAND* and *STEP SIZE* controls **2** are used to control the
voltage-clamp amplifier. The *EPISODES PER TRIAL* selector **3**
is used to define the number of episodes to be acquired,
20   after which the trial is initiated using the *BEGIN* button **4**.
Waveforms are displayed in real-time in the *VOLTAGE and
CURRENT* windows **5** as they are acquired. Journal **6**
automatically logs transcript of experimental session and
provides waveform analysis. The protocol selection area **7**
25   is used to select predefined protocols, while the file/log
management section **8** provides file handling and data output.

The automation routines that may be implemented allow
the entire recording session to be controlled through the
on-screen interface by changing control knobs using the
30   mouse. Automated protocols have been developed that can
initiate and carry out dose-response, reversal potential,
modulator effect and repetitive application experiments with
a single keypress. Waveform analysis routines automatically
measure parameters such as response amplitude, onset time
35   and desensitization time constant and log this information

directly to disk.   Appendix A contains a code listing of the
software implementation which may be operated on computer
10.

Computer 10 may be any computer, computer workstation,
5   dedicated processor, microprocessor or dedicated micro-
controller.   In an embodiment computer 10 is a MacIntosh
IICi computer (Apple Computer; Cupertino, CA), a 68030 based
computer having a minimal configuration of 8 MB RAM and an
80 MB hard drive.   Voltage and current traces are acquired
10   through analog to digital conversion means and similarly
valves and perfusion controllers may be controlled through
digital to analog conversion means.   The analog/digital
conversion means may be, for example, an analog/digital
input output expansion card which may be installed into
15   computer 10.

One preferred analog/digital input/output conversion
means is a MacADIOS II data acquisition card (GW
Instruments; Somerville, MA).   This NuBus based card has an
additional 12 bit A/D converter daughterboard, 2 analog
20   output channels 8-bit digital I/O port, can be configured
with additional daughterboards for enhanced functionality to
facilitate independent acquisition of 2-channel data and a
16 bit digital I/O daughterboard that is used to trigger
digital TTL lines for control of solenoid valves.   The
25   MacADIOS II card is interfaced to laboratory equipment
through a MacADIOS APO analog I/O panel which provides
electrical connections, such as BNC connections, directly to
the card.   As additional channels are needed, one or more
secondary analog/digital converters or additional
30   analog/digital input/output conversion cards may be added.
In one embodiment, a second 12-bit A/D converter was
installed to facilitate independent acquisition of 2-channel
data at high speeds, while a 16-bit digital I/O
daughterboard allowed individual addressing of a total of 24
35   digital output lines for the control of solenoid valves and

other devices. Other data acquisition cards may also be used. While other computers and instrumentation software may be used, the graphical interface of the MacIntosh and the SuperScope II file format simplify manipulation and

5   plotting of waveforms. A 68030 based computer can digitize a 2-channel electrophysiological data at about 100 Hz and recorded directly to 90 MB removable data storage devices (Bernoulli cartridges; Iomega, Inc.) to facilitate convenient storage and retrieval. The implementation of the

10 cellular physiology workstation is not computer specific, as computer technology and storage technology improve, the cellular physiology workstation may be implemented on the improved computer and storage platforms. Functions and protocols on the physiological workstation can be developed

15 as the need arises. Computer programs and data analysis routines not available in the SuperScope II environment can be written in the C programming language for import into the existing virtual instrument.

    Sample data representative of traces produced by a

20 system according to the invention is shown in Figure 7-
    Figure 14. In Figure 7, responses are shown for several ligand-gated ion channels that were expressed after injection of the oocytes with rat brain mRNA. Traces show responses to about 100 μM γ-aminobutyric acid (GABA) (A),

25 about 100 μM kainate (B), and about 100 μM AMPA (C).
    Standard buffer solutions were used with a holding potential of about -100 mV. Automation enables extended recording sessions with minimal operator intervention.

    Figure 8 depicts the results of a test to determine the

30 accuracy of a cellular physiology workstation according to the present invention. In Figure 8, Averaged kainate dose-response data from 4 oocytes injected with GluR6 cRNA which yields an EC_{50} of about 0.5 μM under Vh of about -100 mV,

favorably compares to an EC_{50} of about 1 μM as previously reported.

Figure 9 depicts the effect of pregnenolone sulfate on kainate dose-response curve in oocytes injected with rat brain poly A^+ mRNA. Kainate dose-response curves were generated with and without the neuroactive steroid, pregnenolone sulfate (PS), and under Vh of about -100 mV to determine the mechanism of action. A decrease in Emax suggests a noncompetitive mechanism. This experiment demonstrates the utility of a system according to the invention for performing dose-response experiments with and without modulators.

Figure 10 depicts the measurement of pregnenolone sulfate IC50 in oocytes injected with rat brain poly A^+ mRNA under Vh of about -100 mV. Pregnenolone sulfate was applied in increasing concentrations with 100 μM kainate to characterize its inhibitory effect.

Figure 11 depicts the effects of pregnenolone sulfate and 5β3αS on recombinant GluR6 kainate receptors. All measurements were performed under Vh of about -100 mV. Recombinant receptors may be rapidly characterized by utilization of automated methodologies according to the invention. The inhibitory effects of neuroactive steroids PS and 5β3αS are shown in this experiment. These steroids decrease the maximal response to kainate with no change in kainate EC50.

Figure 12 depicts steroid, pregnenolone sulfate and 5β3αS, IC50 determined for recombinant GluR6 receptors under Vh of about -100 mV. Increasing concentrations of two steroids were applied with 10 μM kainate to determine the steroid IC50.

Figure 13 depicts the kainate concentration dependence of pregnenolone sulfate inhibition. To further characterize the mechanism of inhibition for neuroactive steroids,

pregnenolone sulfate was applied with increasing concentrations of kainate to determine the percent inhibition observed. It was found that concentration dependence of 100 μM pregnenolone sulfate inhibition of

5   kainate induced currents. The currents were expressed as percent change from kainate D-R. Percent change was calculated as $((I_{kain+PS}/I_{kain})-1)\bullet 100$.

Figure 14 depicts inhibition of kainate responses by stimulation of metabotropic glutamate receptors. The

10   ability of a system according to the invention to do repetitive applications of agent solutions was used to make the finding that about 500 μM of the metabotropic agonist, tACPD, can inhibit responses to kainate of about 100 μM in oocytes injected with rat brain poly A^+ mRNA.

15   Detailed specification one implementation of the cellular physiology workstation is listed in Table 1.

Table 1. Parameters of perfusion system and recording chamber of a cellular physiology workstation

Parameter	Measured value
rise time (5% to 95%)	about 70 msec to about 140 msec
solution exchange time (90%)	about 8 second
lag time	about 100 msec
flow rate	about 1.5ml/minute to about 3.0ml/minute
chamber volume	about 75 μl to about 100 μl
dead volume	about 1 μl

Simultaneous Perfusion of Parallel Recording Chambers

The cellular physiology workstation may also be directed to simultaneous recordings from multiple oocytes such as, for example, simultaneous and coordinated perfusion

of two or more recording chambers. Several approaches can be taken to accomplish this objective.

First, valve outputs can be divided into two or more channels using tubing leading into separate manifolds.

5 Solution from each constant-flow chamber flow into a single valve, where it diverges into two or more channels. With this approach, only one set of solutions has to be made up prior to experimentation which reduces the problem of slight differences in concentration obtained from making up

10 multiple, but distinct batches.

Second, the constant-flow chambers are manufactured with multiple output lines. Each output line feeds into a separate valve and, subsequently, into a separate manifold. This approach requires a greater expenditure in valves and

15 associated control circuitry, but minimizes hydrodynamic problems associated with division of solution flow.

Lastly, separate sets of constant-flow chambers can be used for each recording chamber. Though easier to implement, this requires a greater daily expenditure of time

20 required to prepare solutions as well as the expense of additional valves.

Electrophysiological recordings from *Xenopus* oocytes are typically performed using a voltage-clamp amplifier in two-electrode voltage-clamp mode. This method utilizes both

25 a voltage-recording electrode and a current-injecting electrode for the control of membrane voltage. Electrophysiological recordings from additional oocytes may require two additional electrodes per oocyte, as well as appropriate headstages and micromanipulators for positioning

30 of electrodes.

The present design of commercially available voltage-clamp amplifiers, however, only provides inputs for two microelectrode headstages. A multichannel amplifier for electrophysiology can be used to allow simultaneous

recordings from multiple cells. Several approaches can be taken.

First, simultaneous recordings can be based on commercially available voltage-clamp amplifiers. One

5 amplifier and two intracellular electrodes can be used with each recording chamber in a typical recording configuration. Electrophysiological traces are acquired from the multiple amplifiers using the currently existing data acquisition system, as described below, which has the capacity to

10 capture up to 10 channels of analog data.

Low-cost amplifiers that have a smaller size and lack the extensive features found on higher cost amplifiers may also be used. Several of these amplifiers can be combined into a multi-channel device that readily handle simultaneous

15 recordings. Electrophysiological traces from these separate amplifiers would feed into the currently existing data acquisition system.

Another embodiment incorporates an amplifier that is designed to handle multichannel data and facilitate

20 simultaneous recordings. This device can have inputs for up to about 10, or more, microelectrode headstages and the appropriate circuitry for electrode zeroing, bridge balancing and adjustments for capacitative currents and series resistance. Designed from the outset as an amplifier

25 for simultaneous recordings from multiple cells, this instrument is able to acquire multi-channel data at high speed and smoothly integrates into a fully automated system for simultaneous recordings.

Another embodiment is based on designing a novel

30 instrument that accepts input from about 10 microelectrode headstages and feeds the data into standard, commercially available voltage clamp amplifiers. This device would have its own circuitry to maintain the proper holding potential for a given cell while the device is cycling through the

other cells. This type of device eliminates the need and
expense of purchasing multiple amplifiers.

One embodiment of the invention utilizes the MacADIOS
II board by GW Instruments, Inc., for data acquisition and
5   instrument control. This NuBus-based board is configured
with two analog-to-digital converters (ADC) that can acquire
2-channel data at about 25 KHz, but is also capable of
multiplexed data acquisition of about 10 or more channels at
about 3 KHz, which is of sufficient resolution for *Xenopus*
10   oocyte electrophysiology. Thus, the design of the automated
workstation for electrophysiology enables simple scale-up
which may not require additional computer or data
acquisition boards. Electrophysiological traces from the
additional recording chambers would be either displayed in
15   separate windows or superimposed for independent viewing and
analysis.

An application of a cellular physiology workstation
with highly parallel monitoring and perfusion capabilities
is the rapid generation of dose-response data from multiple
20   oocytes. A series of agent solutions of increasing
concentration can be prepared in the constant-flow chambers
of the perfusion system and the lines and manifold primed to
load an agent to be tested. By sweeping the microscope head
across the stage of parallel recording chambers, RNA-
25   injected oocytes are successively positioned in each
recording chamber and impaled with both voltage-recording
and current-injecting microelectrodes. Electrode zeroing,
bridge balancing, and adjustments for capacitative currents
and series resistance are each independently performed as
30   required on each voltage-clamp amplifier. For a typical
dose-response experiment, multiple voltage-clamp amplifiers
are simultaneously stepped to a holding potential
appropriate for the ionic current under study. This can be
accomplished by distributing the output of the digital-to-
35   analog converter (DAC) from the MacADIOS II board to the

separate voltage-clamp amplifiers. The perfusion system may
be set to deliver the desired agent solutions to the
multiple recording chambers simultaneously. Depending upon
experimental design, multiple oocytes may be simultaneously
5   exposed to the same agent to replicated a single experiment
on multiple oocytes, or each oocyte can be exposed to a
different agent to facilitate rapid screening of large drug
libraries. Current and voltage recordings are acquired from
each amplifier and fed into appropriate windows in the
10   SuperScope II virtual instrument. At the conclusion of the
protocol, automated routines perform waveform analysis on
each current recording.

       While the automated electrophysiological workstation is
particularly suited to oocyte electrophysiology, the
15   approach described in general can be readily adapted to
patch-clamp electrophysiology, calcium imaging studies,
confocal microscopy and other applications where perfusion
control and data acquisition need to be tightly integrated.
Any type of biosensor capable of producing an electrical
20   output, such as a sensor capable of measuring concentrations
of substances within the cell, can be used in place of or in
addition to the voltage-measuring electrode. Biosensors are
well known to those of skill in the art and are reviewed for
example by Lowe (Lowe, C.R. Biosensors, Trends in
25   Biotechnology, 2:59-65, 1984) and by Byfield and Abuknesha
(Byfield, M.P., Abuknesha, R.A. Biosensors & Bioelectronics
9:373-400 1994). Other automation aspects that may be
optionally incorporated into the automated cellular
physiology workstation are digitally controlled voltage-
30   clamp amplifiers, and robotics and machine vision to
automate the tasks of oocyte placement and microelectrode
positioning to result in a fully automated
electrophysiological assay system.

       In another embodiment, the present invention relates to
35   a high-throughput, parallel electrophysiology system which

will enable simultaneous recordings from multiple cells.
The embodiment to be discussed here is a 4 recording chamber
embodiment, however, one of skill in the art will recognize
that scaling up to a larger number of recording chambers is
5   a matter of routine experimentation.

In the 4 recording chamber embodiment, the dissecting
microscope discussed in connection with embodiments
described above is replaced with video cameras. A dedicated
camera is positioned above each of the 4 recording chambers.
10   A number of video systems have been evaluated and a
commercially-available, inexpensive color CMOS camera has
proven adequate. The CMOS camera/lens system employed can
give 10-20X magnification at a working distance of about 4
inches. Video images from each of these cameras feed into a
15   commercially-available video controller box that is used to
select between video feeds. For oocyte impalement, the
operator stands in front of a video monitor and a pair of
joysticks which are used to control electronic
micromanipulators. Microelectrode positioning and oocyte
20   impalement are accomplished on the basis of the video image.
The operator can switch between the different video feeds
from the 4 recording chambers with the video controller box,
and can switch between 4 different micromanipulator pairs
with a micromanipulator controller box.
25   Video-enablement will set the stage for full automation
of the electrophysiology process where video images will be
fed into a computer via a frame-grabber card. Image
analysis software will automatically determine where the
microelectrodes are in relation to the oocyte and guide
30   microelectrode positioning and impalement.

Image analysis software is used to identify and outline
the circular shape of the oocyte on the video image. At a
10-20X magnification that the CMOS cameral will enable, the
oocyte will be taking up about 50% of the field of view,
35   allowing for easy identification. Similarly, the glass

microelectrodes will be identified in the video image based on the distinctive outline of the electrodes. The microelectrodes are controlled by commercially-available electronic micromanipulators that are computer controllable.

5   The image analysis software is therefore able to move the electrode tip into an appropriate position above the oocyte, for example, positioning the tips at a point halfway between the oocyte center and edge. In this manner, the microelectrodes will be moved into position directly above

10 the oocyte in an automated manner. This approach can be utilized for either 2 separate microelectrodes, or for a single unit "double-barrel" microelectrode.

At this point, the microelectrodes will be positioned above an oocyte. As the electrode is lowered toward the

15 oocyte, monitoring of the electrode junction potential will allow determination of tip location in the following manner.

Phase	Resistance
Air	Infinite
In solution	Very low
Contacting oocyte	Increasing
Impalement	Low

20

Therefore, automated routines are developed to automate the process of microelectrode descent and oocyte impalement. This phase does not require any video-based information, but

25 electrode impalement can be followed on the video monitor. If desirable, video-based tracking of membrane dimpling that occurs during impalement could be used to provide additional information for automated impalement. The membrane invaginates as the microelectrode pushes inward and then

30 pops back upon penetration. Image analysis software for tracking these events can be developed routinely.

More advanced analysis can also be undertaken. For example, automated testing of cell viability and response can be addressed. Upon impalement, an automated determination can be made if resting potential and membrane resistance fall within acceptable predefined ranges. The impaled oocyte can be perfused and allowed to equilibrate for a predetermined period of time prior to such testing. Control responses can be obtained from the cell to determine if receptor expression levels are within an acceptable range. All these determination are made in an automated manner.

In addition, advanced waveform analysis routine can be developed. Specific analysis modules are developed for analysis of responses from ligand-gated ion channels, voltage-gated ion channels, G-protein coupled receptors, etc. Each module will have routines that will enable the measurement of relevant parameters of a typical response including amplitude, onset time, desensitization rate, etc.

The system is also useful for automated plotting. For example, automatic generation of concentration-response and current-voltage plots on the basis of averaged data from multiple experiments can be enabled in the system. Such features will greatly reduce the time and effort necessary to generate plots from electrophysiology data.

Other embodiments and uses of the invention will be apparent to those skilled in the art from consideration of the specification and practice of the invention disclosed herein. All references cited herein, for whatever reason, are specifically incorporated by reference. The specification and examples should be considered exemplary only with the true scope and spirit of the invention indicated by the following claims.

Examples

Example 1 <u>Oocyte Isolation</u>.

Female, oocyte positive *Xenopus laevis* frogs, purchased from Nasco, Inc. were kept on an about 12 hour light/ about

5   12 hour dark cycle. Frogs were maintained on a diet of chopped calf liver fed about every three days. Prior to surgery, frogs were anesthetized in a solution containing about 0.15 % Tricaine for about 30 minutes. Ovarian sections were removed through a lateral abdominal incision,

10   after which the incision was sutured with about 4 to about 5 stitches and the animal allowed to recover in isolation for about 3 hours to about 4 hours. Ovarian lobules containing follicular oocytes were immediately placed in calcium-free ND96 solution (96 mM NaCl, 1 mM $MgCl_2$, 2 mM KCl, 50 mM

15   Hepes, 2.5 mM pyruvate) and cut into groups of about 10 to about 20 oocytes. Following a treatment with collagenase (Sigma, type II, 2 mg/ml) at about 2 mg/ml for about 2 hours at room temperature, individual oocytes were obtained free of their follicular layer. Selected Dumont stage V and VI

20   oocytes were then transferred to 60 x 15 mm glass petri dishes containing ND96 (96 mM NaCl, 1 mM $MgCl_2$, 2 mM KCl, 50 mM Hepes, 2.5 mM pyruvate) and maintained in an incubator at about 18°C to about 20°C. On the following day, batches of about 20 oocytes to about 30 oocytes were injected with

25   about 30 nl to about 80 nl prepared RNA solution using an electronic microinjector (Drummond Instruments, Inc.).

Example 2 <u>RNA Preparation</u>.

RNA was prepared for injection into oocytes by extraction of mRNA from brain tissue and by synthesis using

30   *in vitro* transcription of linearized DNA templates encoding recombinant receptor subunits.

The extraction technique of RNA preparation uses brain mRNA from chick embryos of about 19 day old as starting material. Extraction was performed using the Dynabeads

Oligo-dT$_{(25)}$) isolation kit (Dynal, Inc.) which utilizes magnetic beads having an attached poly-thymidine oligomer to allow magnetic separation of poly(A)$^+$ RNA from cell homogenates.

5   *In vitro* transcription was performed using plasmids containing the GluR3 (flop) and GluR6 cDNA as starting material. Plasmids were linearized with restriction endonuclease XhoI (GluR3) or XbaI (GluR6) prior to *in vitro* transcription with T3 RNA polymerase using a commercially

10   available kit (Message Machine; Ambion, Inc.; Austin, TX).

Example 3 <u>Electrophysiology</u>.

       About 3 days to about 5 days after RNA injection, electrophysiological recordings were carried out using an Axoclamp-2A voltage clamp amplifier (Axon Instruments,

15   Inc.). Experiments were performed in two-electrode voltage clamp mode using two intracellular microelectrodes of about 1 to about 3 mega-ohm resistance filled with a solution of about 3M KCl. A close-up view of an oocyte under impalement in voltage-clamp mode is shown in Figure 7.

20   Oocytes were usually clamped at a holding potential of about -60 mV and stepped to about -100 mV during agent application. GluR6 injected oocytes were treated for about 10 minutes with a solution of about 10 μg/ml concanavalin A to prevent fast desensitization of kainate responses.

25   Example 4 <u>Application of GABA to Oocytes</u>.

       In this experiment, a solution comprising about 100 μM GABA was applied to an oocyte expressing GABA$_A$ receptors to test the function of the automated workstation. Oocytes were immobilized in the recording chamber, impaled with

30   voltage-recording and current-injecting microelectrodes, and allowed about one to about two minutes to recover to a resting membrane potential of about -40 mV to about -50 mV. The amplifier was switched into voltage clamp mode,

typically at a holding potential of about -60 mV, prior to the start of experimentation protocol.

A typical experimental protocol may comprise the steps of prepulse, agent application, and washout phases and the
5   function of each phase may be set up in advance by defining the positions of on-screen markers M1-M4, which can be easily moved via a user input device such as a computer mouse. The PREPULSE VALVE selector is used to select a prepulse solution which may be used to pre-equilibrate with
10   a modulator before coapplication of modulator and agonist. The modulator is applied during the interval M1 to M2. The DRUG VALVE panel of buttons is next used to select the solution that is to be applied during the interval M2 to M3. Finally, the WASH VALVE selector is used to select the
15   solution to be applied during the washout phase of the protocol, defined as the interval M3 to M4. The WASH VALVE selector also controls the WASH TIMER, which perfuses the oocyte for a preset amount of time between successive episodes.
20   After the perfusion controls were set, the VC COMMAND slider is used to select the voltage offset that will be sent to the amplifier to determine the holding potential at the start of the first episodes. The oocytes were held at about -60 mV and stepped to about -100 mV at the start of
25   data acquisition to increase electrochemical driving forces; this entails a voltage offset being sent to the amplifier of about -40 mV. Experiments usually consist of multiple episodes per trial, where an episode is defined as a single cycle of data acquisition. The EPISODES PER TRIAL selector
30   is used to select the number of episodes to be acquired, after which the experiment is started by pressing the BEGIN button. At the initiation of data acquisition, the holding potential is stepped to the preset voltage, and perfusion commences with the selected solutions. At the end of data
35   acquisition, the current and voltage traces were saved to

disk, and the oocyte is perfused with wash solution for a predetermined amount of time. Each episode is logged to the journal window, where analysis routines measure and log waveform parameters. From this basic protocol, more complex

5   protocols were developed that automatically execute repetitive application, dose-response, reversal potential and voltage-stepping experiments.

Example 5 <u>Repetitive Application Experiments</u>.

      The EPISODES PER TRIAL selector is used to specify a

10   repetitive application protocol, in which multiple cycles of agent application are desired. Figure 15 shows the results from an experiment in which 100 μM GABA was applied repeatedly to an oocyte injected with chick brain poly(A)$^+$ RNA. A protocol of about 30 seconds was used, consisting of

15   a 10 second prepulse with buffer solution, a 10 second application of 100 μM GABA, and a wash phase of about 10 seconds, followed by a wash cycle of about 60 seconds with buffer solution prior to the next application of agent. An increase in current amplitude was evident that reached a

20   plateau over the course of about one hour experiment in which 100 μM GABA was applied 30 times. These experiments demonstrate the utility of the workstation that has been developed, as the experiment is performed automatically without any operator intervention. This type of protocol is

25   useful for following a response over an extended period of time which is useful for looking at time-dependent processes such as rundown of receptor mediated responses. This protocol can also be used to study the effects of compounds that have a slow time course of action, for example,

30   compounds that affect the phosphorylation state of receptors, such as kinase inhibitors or membrane permeant cAMP analogues. Lastly, the repetitive application feature makes it possible to easily compare averaged data taken before and after an experimental manipulation. By avoiding

comparison between single responses, averaging of data reduces errors due to response variability, noise and time-dependent changes in response amplitude.

Example 6 Dose-Response Determination.

5   An automated workstation for electrophysiology was designed to fully automate dose-response experimentation. Typically, the generation of dose-response data is usually achieved by application of increasing concentrations of a given agent to a responsive cell. Figure 8 shows the

10   results of a dose-response experiment in which increasing concentrations of kainate were applied to an oocyte expressing homomeric GluR3 kainate receptors formed from cloned receptor subunits. For the kainate response from this cell, an EC_{50} of about 27 μM was calculated. This type

15   of experiment is initiated by pressing the DOSE/RESPONSE button, which selects a protocol designed to sequentially step through a series of agent solutions, beginning at the valve specified by the ALTERNATE VALVE selector. The EPISODES PER TRIAL selector is then used to specify 12

20   episodes, corresponding to the number of concentrations to be tested. The dose-response protocol closely follows the operation of the repetitive application protocol, with the exception that this protocol increments the DRUG VALVE selection after each episode.

25   Figure 16 shows a high-resolution dose-response curve generated by the Cellular physiology workstation. In this experiment, increasing concentrations of kainate are sequentially applied to an oocyte expressing rat GluR3 receptors. An automated protocol steps through up to about

30   15 different agent concentrations to rapidly generate dose--response curves. An EC_{50} of about 90 μM was determined for GluR3 receptors expressed in *Xenopus* oocytes. Oocyte was held at about -100 mV during kainate application and washed for about 60 seconds between episodes. Total duration of

this experiment, which was performed automatically without operator intervention, was about 15 minutes.

Figure 15 depicts the reproducibility of dose-response curves generated by the cellular physiology workstation.
5   Four separate GABA dose-response determinations were made about 20 minute apart to document system performance. EC_{50} determinations yielded similar results (26 μM, 19 μM, 20 μM, and 21 μM) over the course of this 70 minute experiment. Oocytes were held at about -100 mV during application of
10   about 5 μM, about 10 μM, about 50 μM, about 100 μM and about 500 μM GABA, and washed for about 30 seconds between episodes. Dose-response curves were determined on an oocyte expressing $GABA_A$ receptors after injection with chick brain poly A^+ RNA.

15   In a separate experiment, to determine reproducibility and reliability of agonist responses, the cellular physiology workstation was used to determine current responses to 30 consecutive applications of 100 μM GABA (Figure 17). Current responses to 30 consecutive
20   applications of 100 μM GABA are shown in an oocyte expressing $GABA_A$ receptors after injection with chick brain poly A^+ RNA. Slight increases in current amplitude are observed during the course of this 45 minute experiment, which was performed automatically without operator
25   intervention. Oocytes were held at about -100 mV during agent application and washed for about 1 minute with Ringer solution between each of the 30 episodes.

Example 7 Reversal Potential Determination.

The virtual instrumentation that was developed also
30   provides control over the voltage-clamp amplifier, thereby making it possible to automate experiments in which holding potential may be varied, such as those determining reversal potentials and examining current-voltage relationships. A reversal potential for a receptor-mediated response is the

voltage at which no net current is observed upon activation
of the ionic conductances associated with the receptor.
Reversal potentials are typically determined by applying a
given agent at various holding potentials, plotting current
5   vs. holding potential, and calculating the voltage at which
the current reverses direction. Figure 18 shows the results
of an experiment in which a reversal potential of about -15
mV to about -10 mV was determined for the kainate response
in oocytes expressing GluR6 kainate receptors. The holding
10   potential was progressively increased from about -80 mV to
about +25 mV in 11 steps of about 10 mV. The oocyte was
returned to a holding potential of -60 mV and washed for
about 30 second after each agent application. The direction
of the kainate-induced current was found to reverse between
15   about -15 mV and about -10 mV. This type of automated
protocol can also be used to determine current-voltage
relationships, in the absence and presence of a receptor
modulator and to investigate mechanisms of action of
modulatory drugs. For these types of voltage experiments,
20   the STEP SIZE slider is used with a multi-episode trial to
increment the VC COMMAND offset, which controls the holding
potential of the amplifier.

Example 8 Voltage-Stepping Response.
     The examples described above used a perfusion system
25   for applying a receptor ligand during the period between the
M2 and M3 markers to generate a receptor-mediated response.
The cellular physiology workstation may also be programmed
for examining voltage-gated ion channels. For example, the
Xenopus oocyte membrane has a number of well characterized
30   voltage-dependent conductances, including an endogenous
chloride current ($I_{c1}-$), an endogenous calcium-dependent
chloride current ($I_{Cl}-_{(Ca++)}$), and an endogenous sodium current
(I_{Na+}), that can sometimes interfere with other currents of
interest and may sometimes be subtracted out. Figure 19

shows traces from an experiment using the cellular
physiology workstation to examine endogenous, calcium-
dependent chloride current ($I_{Cl^-(Ca++)}$) found in native *Xenopus*
oocytes. Voltage was stepped from about -100 mV to about

5   +20 mV, each time returning to a holding potential of about
-60 mV, to determine the current-voltage relationship for
this conductance. In these types of experiments, voltage is
stepped from thy amplifier's holding potential to a voltage
determined by the VC COMMAND and STEP SIZE sliders during

10 the M2-M3 interval. Predefined protocols are in this way
established for voltage-dependent conductances of interest,
and can be easily selected through on-screen buttons. This
type of protocol can be used to screen recombinant voltage-
dependent ion channels against libraries of agents such as

15 drug libraries.

Outlined herein is a set of hardware and software for
high-throughput, parallel electrophysiology enabling
simultaneous recordings from multiple cells. The software
will be developed using the LabVIEW (TM) Professional

20 Development System, Version 6i for Windows 95/98/2000/NT.
The completed application package to be delivered will
consist of all source code VIs along with compiled
executable modules. The Professional Development System
includes an Application Builder, which is used to create

25 stand-alone executable routines that may be distributed
among several PCs without the need for additional site or
software licenses. The hardware proposed herein is based on
a combination of National Instrument's E-series plug-in PCI-
bus data acquisition boards (for analog inputs and outputs)

30 and FieldPoint products (for support of the digital outputs
for switching solenoid valves). This combination of
installed and remote hardware optimizes the cost/performance
tradeoffs for each kind of functionality. The installed PCI
boards can stream data continuously through buffered

35 acquisition at the desired maximum 3 kS/sec, while the

FieldPoint modules allow for the higher channel counts and somewhat slower response times required for solenoid valve controls. For this application, communications between the host PC and FieldPoint modules will be accomplished via an
5   RS-485 serial link.

The host PC will be housed in a standard 19-inch laboratory equipment rack. Optimally, the FieldPoint modules are located in close mechanical proximity to the respective solenoid valves they control in or to minimize
10   cabling complexity.

The system proposed will facilitate a variety of functions. For example, when first entering the program a data acquisition server engine will be launched and will run as a background task. The purpose of this server engine is
15   to acquire data continuously into a buffer at the maximum required sampling rate, and serve contiguous slices of that buffered data as requested to individual cell software modules. A menu screen is displayed on the host PC. The menu screen prompts a user to select a desired cell (e.g.,
20   Cell 1-4) to configure or operate and may use this screen as a central navigation point from which to access any running cell software module. In this manner, individual cell software modules may run independently and asychronously of one another, and data may be collected at different rates as
25   desired.

The menu screen discussed above may contain a button entitled "Go To Recipe Editor" which will enable customization of an experimental protocol. Each step in an experimental protocol is defined by which of a variety of
30   solenoid valves are open or closed, the resting and testing voltage clamp potential, and the respective duration of the three phases (prepulse, drug application, and washout) which comprise each episode. An appropriately created Recipe file that delineates each of these values will control operation
35   of the hardware during a given experimental run. The

creation and editing of these recipe files is accomplished through the use of a Recipe Editor VI, whose font panel is similar to that shown in Fig. 21. For each discrete parameter, the user simple clicks on the desired checkbox to

5   select the particular function. Various set points and tolerances are entered through numerical controls. Intuitive editing commands (e.g., insert, delete, go to next step, go to previous step) are provided. When a new step is inserted, all values in that step are initially copied from

10 the previous step. The used can edit such settings as desired. In this manner, steps which reflect a small change from a previous operation are easily created with minimum effort. Note that provision has been made to accommodate up to 100 solenoid valves, any combination of which may be

15 turned on during the application phase. New recipes may be created from scratch, old recipes may be loaded and edited, and any recipe created may be stored to file for later recall and use from a running cell module.

        Whenever the system is following a recipe and

20 performing an automated sequence of operations, the main display screen will be displayed. This main display screen is similar to embodiments described previously. Various mode control buttons, which allow the user to start, pause or abort the current process, are provided. The Operating

25 Screen shows all present values of the parameters in the system (e.g., valve open/closed status, amplifier voltage set point, collected voltage and current waveforms) and the results of analysis such as peak amplitude and rise time. Raw voltage and current data are saved as a delimited text

30 file for easy import into spreadsheets and analysis packages. Each cell is accessed from a Start Screen. A further description of the display functions and modes is listed in the Specification which follows at the end of this description.

As previously described, a combination of National Instruments' PCI plug-in data acquisition boards and FieldPoint hardware will serve as the primary data acquisition and control interfaces between the host computer

5 and the actual process elements. For each host PC, a PCI-6034E (16-bit, 16 channel input card) and a PCI-6703 (16-bit, 32 channel output card) are installed. A nominal count of four cells per host PC is initially targeted, with the potential for future expansion as practical. The

10 requirement of 2 analog inputs per cell dictates that for each installed PCI-6034E card, a maximum of 8 cells may be accommodated. Wiring to and from these PC cards shall be accomplished though the use of shielded cable assemblies and related modular screw-terminal blocks, which facilitate easy

15 wiring and servicing. The initial FieldPoint physical configuration will consist of one "bank" of six FP-DO-403 16-channel current-sinking output modules, controlled by (and communicating to the server computer with) an associated Network Module. One bank of modules will provide

20 96 discrete outputs, sufficient for four cells up to 24 solenoid valves per cell. Additional solenoid valves, as well as future expansion to higher cells per host PC, can be accommodated in a modular manner by adding further banks of Network and Output modules. These banks are DIN-rail

25 mountable in any convenient location. We envision that an appropriate instrumentation cabinet will be employed to house both the FieldPoint control elements as well as the associated solenoid valves. Each bank will be powered by a dedicated modular DC power supply.

30 This project requires the use of an appropriately modern and powerful PC to serve as a host platform. At a minimum, this PC must have the following specifications:

Processor: 600 MHz Pentium® III

	Memory:	256 MB 100 MHz SDRAM or more
	Hard Drive:	20 GB or larger
	Monitor:	17" or larger, 0.28dp
5	Video Card:	AGP graphics card, 8 MB or more
	Network Card:	3Com C905C TXM 10/100 or equivalent
	Modem:	V.90/56K PCI DataFax
10		Modem or equivalent
	CD-ROM:	48X max variable CD-ROM Drive
	Re-writable Large Media:	100 MB Zip Drive or CD-RW Drive
15	Installed Shrink-wrap Software:	Windows 98 2E/2000/NT 4.0
	Recommended:	Office 2000
		McAfee VirusScan 4.02
		PcAnywhere 32 v9.0

Specifications

20   A minimal implementation of the automated parallel electrophysiology system would consist of the following:

- An initial design that would enable the control of 4 separate chambers. The modular software design should be easily scalable as the number of chambers is
25   increased from 4 to 100.
- The ability to acquire, display, and save current and voltage waveforms from multiple chambers at between 300-3000 Hz for each waveform for a period between 1 and 600 seconds. We would want to be able to view, in
30   real-time, simultaneous traces from all amplifiers on screen as they are acquired.
- Ability to control 4 separate banks of solenoid valves, each bank initially consisting of 20 valves, expandable

to 100 valves. Each bank would be addressable as to which valve is turned on during periods that would be set by on-screen movable markers. Each individual drug application is divided into three phases: 1) pre-application, 2) application, and 3) wash-out, each of which may be of varying duration (which may be zero). Independent control of the solution (valve) and duration is required for each of these three phases. In addition, it should be possible to program one or more changes in holding potential to an operator determined potential, via a command to the appropriate amplifier at any time during any of these phases, by placement of an on-screen marker on some kind of timeline or graph. After wash-out, the cell is returned to its resting potential and allowed time to recover during the washing phase, during which it is perfused with buffer for a period specified by separate wash timers (0-240 sec) for each chamber.

o Basic waveform analysis initially consisting of amplitude measured from peak response to an averaged baseline for each response.

o Generation of a textual experiment log that would automatically log the experimental protocol similar to that seen in the existing implementation. A log should be generated indicating the sequence and times of drug application. There should be a method for the operator to insert comments into the log. The log should be saved to disk after each drug application. A mechanism should be provided for the operator to enter labels to identify specific drug solutions (valves). A method should be provided for saving all settings to disk, including operator comments.

o On-screen controls for the control of 4 separate voltage-clamp amplifiers at different voltages if necessary.

o   Built-in protocols for **dose-response** and **voltage-stepping** experiments (described in previous materials).
Dose-response protocols should include the ability to
specify the sequence of solution applications (valves

5   to be activated): increasing numerical sequence,
decreasing sequence, or an arbitrary, operator-defined
sequence. As well, an automated method should be
provided for repeating out a "standard" treatment
between each test treatment. For example, a dose

10   response study might involve the following sequence:
1, 2, 1, 3, 1, 4, 1, 5, 1...(where the numbers denote
specific solutions/valves).

o   Ability to do the same experiment in parallel. We
would also want to be able to easily do the same

15   protocol simultaneously on all 4 chambers. In such a
manner, for example, we could do 4 simultaneous dose-
response experiments on 4 cells. In such a case, the
same set of drug reservoirs would feed into the 4 sets
of solenoid valves.

20   o   A method for taking one or more chambers off-line, and
suspending drug applications to those chambers while
continuing to conduct experiments with the remaining
preparations. As well, we would want to be able to
abort for each chamber in real-time if the cell goes

25   bad, as we wouldn't want to be wasting drugs and using
up disk space on data from a dead oocyte.

o   A list of all the data acquisition hardware and
software that will be necessary for this project.

Other embodiments and uses of the invention will be

30   apparent to those skilled in the art from consideration of
the specification and practice of the invention disclosed
herein. All references cited herein, for whatever reason,
are specifically incorporated by reference. The
specification and examples should be considered exemplary

only with the true scope and spirit of the invention indicated by the following claims.

APPENDIX A

• •

INSTRUMENT • INSTRUMENT • INSTRUMENT • INSTRUMENT • INSTRUMENT • INSTRUMENT

Name: oocyte.77

5   Application: SuperScope II
Version: 1.43 (68k)

• •

DIGITIZER • DIGITIZER • DIGITIZER • DIGITIZER • DIGITIZER • DIGITIZER • DIGITIZER

Digitizer: MacADIOS II
10   Driver: Inout
Data Collection Mode: Chart
Trigger Mode: none
Timebase: 600 pts/trace, 100.00 pts/sec

• •

15   DISPLAYS • DISPLAYS • DISPLAYS • DISPLAYS • DISPLAYS • DISPLAYS • DISPLAYS

Display Name

Current
Voltage

20   •

MARKERS • MARKERS • MARKERS • MARKERS • MARKERS • MARKERS • MARKERS

Marker Name

M1
25   M2
M3
M4

• •

JOURNALS • JOURNALS • JOURNALS • JOURNALS • JOURNALS • JOURNALS • JOURNALS

Journal Name

Journal 1
5   jHeader

•••

STRINGS • STRINGS • STRINGS • STRINGS • STRINGS • STRINGS • STRINGS • STRINGS

Name	Units	Text
retValue	Volts	7
message	Volts	2.12
sAmplitu	Volts	67Ø nA
sVwash	Volts	Ø /
sTrace	Volts	Trace # 128
sVdrug	Volts	
sTime	Volts	at 16:Ø4:32
sVwash2	Volts	Ø
sVoffset	Volts	Vh offset = 12Ø mV
sM2	Volts	M2 = 2.Ø
sM3	Volts	, M3 = 4.Ø
sM4	Volts	, M4 = 6.Ø
sWash	Volts	Wash Ø sec
sAbort	Volts	
_SaveWav	Volts	8/29/95.AD1.128
_LoadWav	Volts	8/29/95.AD1.1Ø3
S15	Volts	
sMax	Volts	3Ø9.6 nA at 4.Ø3 s
wTmax	sec	4.Ø3

•••

30   WAVES • WAVES • WAVES • WAVES • WAVES • WAVES • WAVES • WAVES • WAVES • WAVES

Wave Name	Wave Type	V Units	H Units	Length	ValidPts
ADØ	Integer	Volt	sec	Ø	Ø
AD1	Integer	A	sec	Ø	Ø
Dout	Integer	Volt	sec	Ø	Ø

	seg2.4	Integer	A	sec	0	0
	DA0	Integer	Volt	sec	0	0
	Selected	Float	Volt	sec	0	0
	Flip	Float	A	sec	0	0
5	Dseg2.3	Integer	Volt	sec	0	0
	W1	Integer	A	sec	0	0
	W2	Integer	A	sec	0	0
	W3	Integer	A	sec	0	0
	W4	Integer	A	sec	0	0
10	W5	Integer	A	sec	0	0
	W6	Integer	A	sec	0	0
	W7	Integer	A	sec	0	0
	seg3.4	Float	A	sec	0	0
	Vseg2.3	Integer	Volt	sec	0	0

15 ••• Wave End Points •••

Wave Name	1st Point H Value	Last Point H Value	Sample Period
AD0	0.000000	-0.01000	0.010000
AD1	0.000000	-0.01000	0.010000
Dout	0.000000	-0.01000	0.010000
seg2.4	-0.010000	-0.02000	0.010000
DA0	0.000000	-0.01000	0.010000
Selected	17.279999	17.278999	0.001000
Flip	2.010000	2.00000	0.010000
Dseg2.3	-0.010000	-0.02000	0.010000
W1	0.000000	-0.01000	0.010000
W2	0.000000	-0.01000	0.010000
W3	0.000000	-0.01000	0.010000
W4	0.000000	-0.01000	0.010000
W5	0.000000	-0.01000	0.010000
W6	0.000000	-0.01000	0.010000
W7	0.000000	-0.01000	0.010000
seg3.4	2.000000	1.990000	0.010000
Vseg2.3	-0.010000	-0.02000	0.010000

(Row labels 20, 25, 30 appear in left margin at AD0-Dout, Dseg2.3, W5 rows respectively.)

35 ••• Wave Mapping For 16 bit Integer Waves •••

Wave Name	Quanta	Min Code	Max Code	Min Value	Max Value

AD0	0.000488	-2048	2047	-1.000000	0.999512
AD1	0.000000	0	4095	-0.000001	0.000001
Dout	1.000000	-32768	32767	-32768.000000	32767.000000
seg2.4	0.000000	0	4095	-0.000001	0.000001
DA0	0.004883	-2 048	2048	-10.000000	10.000000
Dseg2.3	1.000000	-32768	32767	-32768.000000	32767.000000
W1	0.000000	0	4095	-0.000001	0.000001
W2	0.000000	0	4095	-0.000001	0.000001
W3	0.000000	0	4095	-0.000001	0.000001
W4	0.000000	0	4095	-0.000001	0.000001
W5	0.000000	0	4095	-0.000001	0.000001
W6	0.000000	0	4095	-0.000001	0.000001
W7	0.000000	0	4095	-0.000001	0.000001
Vseg2.3	0.004883	-2048	2048	-10.000000	10.000000

••• Segments •••

Segment	Parant	M1 Name	M2 Name	M1 Time	M2 Time
seg2.4	AD1	M2	M4	10.000000	30.000000
Dseg2.3	Dout	M2	M3	10.000000	20.000000
seg3.4	Flip	M3	M4	20.000000	30.000000
Vseg2.3	DA0	M2	M3	10.000000	20.000000

••• First 5 Points •••

Wave Name	Point #1	Point #2	Point #3	Point #4	Point #5
AD0					
AD1					
Dout					
seg2.4					
DA0					
Selected					
Flip					
Dseg2.3					
W1					
W2					
W3					

W4

W5

W6

W7

5   seg3.4

Vseg2.3

•••

VARIABLES • VARIABLES • VARIABLES • VARIABLES • VARIABLES • VARIABLES

Name	Units	Value
10	----	----	----
error	Volts	1.000000
V1	Volts	10.000000
V2	Volts	0.000000
V3	Volts	0.000000
15	V4	Volts	0.000000
val.dio	Volts	145.000000
Dout		1.000000
Dport		0.000000
n0	Volts	0.000000
20	n1	Volts	0.000000
n2	Volts	0.000000
n3	Volts	0.000000
n4	Volts	0.000000
n5	Volts	0.000000
25	n6	Volts	0.000000
n7	Volts	0.000000
n8	Volts	0.000000
n9	Volts	0.000000
n10	Volts	0.000000
30	n11	Volts	0.000000
n12	Volts	0.000000
n13	Volts	0.000000
n14	Volts	0.000000
n15	Volts	0.000000
35	secs	Volts	0.000000
cnt1	Volts	0.000000
Vwash	Volts	1.000000
WashOut	Volts	-2.000000

	cnt2	Volts	4.000000
	vOut	Volts	0.000000
	Marker4	Volts	30.000000
	_Tracesi	Volts	2999.999756
5	_SampleP	Volts	0.010000
	vVCstep	Volts	40.000000
	NewDAC	Volts	140.000000
	selected	Volts	1.000000
	cnt3	Volts	5.000000
10	timeV	Volts	0.400000
	V39	Volts	0.000000
	V40	Volts	6.000000
	V41	Volts	24.000000
	wMax	A	309.570312
15	wMin	A	-360.839844
	wAmplitu	A	670.410156
	tracenum	Volts	0.000000
	Marker3	Volts	4.000000
	Marker2	Volts	2.000000
20	vDelSeg	Volts	5.930000
	previous	Volts	0.000000
	V50	Volts	0.903090
	V51	Volts	0.301030
	cnt4	Volts	3.000000
25	flushlp	Volts	4.000000
	Vflush	Volts	16.000000
	FlushOut	Volts	-17.000000
	loadloop	Volts	7.000000
	V57	Volts	103.000000
30	V58	Volts	7.000000
	V59	Volts	109.000000
	vVCcom	Volts	100.000000

• •

DATAPIPES • DATAPIPES • DATAPIPES • DATAPIPES • DATAPIPES • DATAPIPES • DATAPIPES

35	Name	Pathname
	--	
	Datapipe	Mac IIci 80HD:Technical:SuperScope II:Data:8/29/95:

Name	FileName	VolumeName	DirID	vRefNum	WDRef
Datapipe	.AD1.103	Mac IIci 80HD	145109	-1	-32477

• •

5   TASKS • TASKS • TASKS • TASKS • TASKS • TASKS • TASKS • TASKS • TASKS • TASKS • TASKS

Task:wash   Allow mouse activity

Task Begin

String sAbort = "" appended onto end of string ""

10   Variable WashOut = Variable Vwash + 1.000

Variable WashOut = Variable WashOut * -1.00

If (Control bpurge = 1.000) then ...

       Jump to subroutine "synth"

       Variable WashOut = Variable WashOut * -1.00

15   If end

If (Control bHold = 0.00000) then ...

       Cwrite (devType147, offset=192, slot=4; WashOut to Value)

       If end

Variable secs = Control cWashtim * 60.00

20   Control iTimer .1 = 0.00000

While (Control iTimer .1 < Variable secs) do ...

       Synchronize to 1.00000 second intervals

       Control iTimer .1 = Control iTimer .1 + 1.000

       While end

25   Cwrite (devTye147, offset=192, slot=4; -1.00 to Value)

Beep

If (Control bPurge = 0.00000) then

       String sWash = "Variable secs" insert into "Wash sec " after char 5 (base 1)

       String sWash = "String sAbort" appended onto end of string "String sWash"

30   Append text onto the end of Journal1 with data from string sWash

       Insert "

" into journal Journal1

       Insert "

" into journal Journal1

35   If end

Control bPurge = 0.00000

```
Task:abort                                    Allow mouse activity
Task Begin
String sAbort = "Control iTimer .1" insert into "aborted at sec" after char 11
Control iTimer .1 = Variable secs
Variable cnt2 = Control dEpiNum
Break out of Trace loop


-------------------------------------------------------------------------------

Task:acquire                                  Allow mouse activity
Task Begin
Variable cnt2 = 0.00000
Variable vVCcom = Control cVCcom
Jump to subroutine "duration"
Jump to subroutine "header"
Trace Loop Begin (30 traces)
        Control epicnt = Variable cnt2 + 1.000
        Jump to subroutine "synth"
        Variable tracenum = Variable tracenum + 1.000
        Digitize Trace (600 pts/trace, 100.00 pts/sec)
        Jump to subroutine "smooth"
        If (Control bAutosav == 0.00000) then ...
                Jump to subroutine "save"
                If end
        Jump to subroutine "analyze"
        Variable vOut = 0.00000
        Analog & Digital I/O (vOut to Aout0)
        Control bWash = 1.000
        Jump to subroutine "wash"
        Control bWash = 0.00000
        Variable cnt2 = Variable cnt2 + 1.000
        If (Control cVCstep != 0.00000) then ...
                If (Control dEpiNum > 1.000) then ...
                        Variable vVCStep = Variable cnt2 * Control cVCstep
                        Variable NewDAC = Variable vVCcom + Variable vVCstep
                        Variable vOut = Variable NewDAC / 20.00
                        Control cVCcom = Control cVCcom +Control cVCstep
                        Fill wave 'DA0' with 0.00000, 24000 points
                        If (Control bMarkers == 1.000) then ...
                                Vseg2.3 = Vseg2.3 + variable vOut
                                If end
```

Line numbers in left margin: 5, 10, 15, 20, 25, 30, 35

If (Control bMarkers == 0.00000) then ...

    DA0 = DA0 + variable vOut

    If end

  If end

5   If end

If (Variable cnt2 >= Control dEpiNum) then ...

  Variable vOut = 0.00000

  Analog & Digital I/O (vOut to Aout0)

  Break out of Trace Loop

10   If end

Clear & Update

Trace Loop End

Cwrite (devType147, offset=192, slot=4; -1.00 to Value)

Control epicnt = 1.000

15   Jump to subroutine "footer"

Task:synth   Allow mouse activity

Task Begin

Fill wave 'AD0' with 0.00000, 24000 points

20   Fill wave 'AD1' with 0.00000, 24000 points

If (Control bDMD == 1.000) then ...

  If (Variable cnt2 == 2.000) then ...

    Variable V50 = Log10 (Variable previous)

    Variable V51 = Log10 (2.000)

25   Variable selected = Varible V50 / Variable V51

    Variable selected = Int (Variable selected)

    Jump to subroutine "ButonSet"

    If end

  If end

30   Variable n0 = Control V0 * 1.000

Variable n1 = Control V1 * 2.000

Variable n2 = Control V2 * 4.000

Variable n3 = Control V3 * 8.000

Variable n4 = Control V4 * 16.00

35   Variable n5 = Control V5 * 32.00

Variable n6 = Control V6 * 64.00

Variable n7 = Control V7 * 128.0

Variable n8 = Control V8 * 256.0

Variable n9 = Control V9 * 512.0

Variable n10 = Control V10 * 1024

Variable n11 = Control V11 * 2048

Variable n12 = Control V12 * 4096

Variable n13 = Control V13 * 8192

5   Variable n14 = Control V14 * 16384

Variable n15 = Control V15 * 32768

Variable Dport = 0.00000

Variable Dport = Variable Dport + Variable n0

Variable Dport = Variable Dport + Variable n1

10   Variable Dport = Variable Dport + Variable n2

Variable Dport = Variable Dport + Variable n3

Variable Dport = Variable Dport + Variable n4

Variable Dport = Variable Dport + Variable n5

Variable Dport = Variable Dport + Variable n6

15   Variable Dport = Variable Dport + Variable n7

Variable Dport = Variable Dport + Variable n8

Variable Dport = Variable Dport + Variable n9

Variable Dport = Variable Dport + Variable n10

Variable Dport = Variable Dport + Variable n11

20   Variable Dport = Variable Dport + Variable n12

Variable Dport = Variable Dport + Variable n13

Variable Dport = Variable Dport + Variable n14

Variable Dport = Variable Dport + Variable n15

Variable previous = Variable Dport

25   Variable WashOut = Variable Dport + 1.000

If (Control bDR == 1.000) then ...

    Variable selected = Control dAltvalv + Variable cnt2

    Jump to subroutine "ButonSet"

    Variable cnt3 = 0.00000

30   Variable Dport = 1.000

    While (Variable cnt3 < Variable selected) do ...

        Variable Dport = Variable Dport * 2.000

        Variable cnt3 = Variable cnt3 + 1.000

        While end`

35   If end

If (Control bDMD == 1.000) then ...

    If (Variable cnt2 == 1.000) then ...

        Variable selected = Control dAltvalv

        Jump to subroutine "ButsonSet"

40   Variable cnt3 = 0.00000

Variable Dport = 1.000

While (Variable cnt3 < Control dAltvalv) do ...

    Variable Dport = Variable Dport * 2.000

    Variable cnt3 = Varible cnt3 + 1.000

5   While end

   If end

  If end

Variable Dout = Variable Dport + 1.000

Fill wave 'Dout' with -1.0000, 24000 points

10   Dout = Dout - variable Vwash

Dseg2.3 = Dseg2.3 + 1.00000

Dseg2.3 = Dseg2.3 + variable Vwash

Dseg2.3 = Dseg2.3 - variable Dout

If (Variable cnt2 == 0.00000) then ...

15   Fill wave 'DA0' with 0.00000, 24000 points

   Variable vOut = Control cVCcom / 20.00

   If (Control bMarkers == 1.000) then ...

      Vseg2.3 = Vseg2.3 + variable vOut

   If end

20   If (Contrl bMarkers == 0.00000) then ...

      DA0 = DA0 + variable vOut

   If end

   If end

----

25   Task:washvalv   Allow mouse activity

Task Begin

Variable Vwash = 1.000

Variable cnt1 = 0.00000

While (Variable cnt1 < Control dWashVlv) do ...

30   Varible Vwash = Variable Vwash * 2.000

   Variable cnt1 = Variable cnt1 + 1.000

   While end

Task:timebase   Allow mouse activity

35   Task Begin

Marker M4 & wave AD0 intersection (time to Marker4)

Variable _SampleP = 0.01000

Variable _TraceSi = Variable Marker4 * Variable _SampleP

Variable _TraceSi = Variable _TraceSi * 10000

Task:print   Allow mouse activity

Task Begin

5   Choose Print_ Under File

Task:save   Allow mouse activity

Task Begin

String _SaveWav = "" appended onto end of string ""

10   String _SaveWav = date (e.g. "1/30/64")

String _SaveWav = ".AD1." appended onto end of string "String _SaveWav"

If (Variable tracenum < 10.00) then ...

       String _SaveWav = "0" appended onto end of string "String _SaveWav"

       If end

15   If (Variable tracenum < 100.0) then ...

       String _SaveWav = "0" appended onto end of string "String _SaveWav"

       If end

String _SaveWav = "Variable tracenum" appended onto end of string "String _Save

Save wave AD1 to disk

20   ---

Task:setbuton   Allow mouse activity

Task Begin

Control V0 = 0.00000

Control V1 = 0.00000

25   Control V2 = 0.00000

Control V3 = 0.00000

Control V4 = 0.00000

Control V5 = 0.00000

Control V6 = 0.00000

30   Control V7 = 0.00000

Control V8 = 0.00000

Control V9 = 0.00000

Control V10 = 0.00000

Control V11 = 0.00000

35   Control V12 = 0.00000

Control V13 = 0.00000

Control V14 = 0.00000

Control V15 = 0.00000

If (Variable selected == 0.00000) then ...

 Control V0 = 1.000

 If end

5 If (Variable selected == 1.000) then ...

 Control V1 = 1.000

 If end

If (Variable selected == 2.000) then ...

 Control V2 = 1.000

10 If end

If (Variable selected == 3.000) then ...

 Control V3 = 1.000

 If end

If (Variable selected == 4.000) then ...

15 Control V4 = 1.000

 If end

If (Variable selected == 5.000) then ...

 Control V5 = 1.000

 If end

20 If (Variable selected == 6.000) then ...

 Control V6 = 1.000

 If end

If (Variable selected == 7.000) then ...

 Control V7 = 1.000

25 If end

If (Variable selected == 8.000) then ...

 Control V8 = 1.000

 If end

If (Variable selected == 9.000) then ...

30 Control V9 = 1.000

 If end

If (Variable selected == 10.00) then ...

 Control V10 = 1.000

 If end

35 If (Variable selected == 11.00) then ...

 Control V11 = 1.000

 If end

If (Variable selected == 12.00) then ...

 Control V12 = 1.000

40 If end

If (Variable selected == 13.00) then ...

Control V13 = 1.000

If end

If (Variable selected == 14.00) then ...

Control V14 = 1.000

5   If end

If (Variable selected == 15.00) then ...

Control V15 = 1.000

If end

----

10   Task:bDRtask   Allow mouse activity

Task Begin

Variable selected = Control dAltvalv

Jump to subroutine "ButonSet"

Control bDMD = 0.00000

15   Control cVStep = 0.00000

Control sAuto = 0.00000

Task:revpot   Allow mouse activity

Task Begin

20   Control dEpiNum = 5.000

Control cVStep = 10.00

Control cVcom = 30.00

Task:duration   Allow mouse activity

25   Task Begin .

Variable V39 = Control cWashtim * 60.00

Variable V40 = Variable Marker4 + Variable V39

Variable V41 = Variable V40 * Control dEpiNum

Variable timeV = Variable V41 / 60.00

30   Control iTime = Variable timeV

Task:bDMDtask   Allow mouse activity

Task Begin

Control bDR = 0.00000

35   Control cVStep = 0.00000

Control dEpiNum = 3.000

Control sAuto = 0.00000

Task:purge   Allow mouse activity

Task Begin

5   Control bDR = 0.00000

Jump to subroutine "wash"

Task:analyze   Allow mouse activity

Task Begin

10   Flip = seg2.4 * -1.0e+9

Pulse analysis on Flip (min to wMin; max to wMax; tmax to wTmax)

String sTrace = "Variable tracenum" appended onto end of string "Trace # "

Append text onto the end of Journal1 with daa from string sTrace

String sTime = time (e.g. "10:31:02")

15   String sTime = " at " insert into "String sTime" after char 0 (base 1)

Append text onto the end of Journal1 with data from string sTime

Insert "

" into journal Journal1

Insert "Valve" into journal Journal1

20   String sVwash = " / " appended onto end of string "Control dWashV1v"

Append text onto the end of Journal1 with data from string sVwash

Jumpt to subroutine "valvenum"

Append text onto the end of Journal1 with data from string sVdrug

Insert "/Valve" into journal Journal1

25   String sVwash 2 = "" appended onto end of string "Control dWashV1v"

Append text onto the end of Journal1 with data from string sVwash2

Insert "

" into journal Journal1

Marker M2 & wave AD1 intersection (time to Marker 2)

30   Marker M3 & wave AD1 intersection (time to Marker 3)

Marker M4 & wave AD1 intersection (time to Marker 4)

String sM2 = "Variable Marker 2" rounded off to 1 digits after decimal

String sM2 = "M2 = " insert into "String sM2" after char 0 (base 1)

String sM3 = "Variable Marker3" rounded off to 1 digits after decimal

35   String sM3 = ", M3 = " insert into "String sM3" after char 0 (base 1)

String sM4 = "Variable Marker4" rounded off to 1 digits after decimal

String sM4 = ", M4 = " insert into "String sM4" after char 0 (base 1)

Append text onto the end of Journal1 with data from string sM2

Append text onto the end of Journal1 with data from string sM3

Append text onto the end of Journal1 with data from string sM4

Insert "

5   " into journal Journal1

String sVoffset = "Control cVcom" appended onto end of string "Vh offset = "

String sVoffset = "mV" appended onto end of string "String sVoffset"

Append text onto the end of Journal1 with data from string sVoffset

Insert "

10   " into journal Journal1

Insert "Maximum = " into journal Journal1

String sMax = Variable wMax

String sMax = "String sMax" rounded off to 1 digits after decimal

String sMax = " nA at " appended onto end of string "String sMax"

15   String wTmax = "String wTmax" rounded off to 2 digits after decimal

String sMax = "String wTmax" appended onto end of string "String sMax"

String sMax = "s" appended onto end of string "String sMax"

Append text onto the end of Journal1 with data from string sMax

Insert "

20   "into journal Journal1

Variable wAmplitu = Variable wMax - Variable wMin

If (Control sGain == 1.000) then . . .

    Variable wAmplitu = Variable wAmplitu * 10.00

    If end

25   String sAmplitu = Variable wAmplitu

String sAmplitu = "String sAmplitu" rounded off to 0 digits after decimal

String sAmplitu = "nA" appended onto end of string "String sAmplitu"

Insert "Amplitude = " into journal Journal1

Append text onto the end of Journal1 with data from string sAmplitu

30   Insert "

" into journal Journal1

Insert "

" into journal Journal1

----

35   Task:overlay   Allow mouse activity

Task Begin

Variable V58 = Control dEpiNum

Variable V59 = Variable tracenum

Show Alert: "Overlay data from last trial?"

If (Variable error == 2.000) then . . .

    Show Alert: "Start with what episode?"

    Variable V59 = String retValue

5   Show Alert: "Overlay how many previous traces (2-7)?"

    Variable V58 = String retValue

    If end

Variable loadloop = 0.00000

If (Variable loadloop < Variable V58) then . . .

10   Jump to subroutine "MakeLoad"

    Load wave W1 from disk

    If end

If (Variable loadloop < Variable V58) then . . .

    Jump to subroutine "MakeLoad"

15   Load wave W2 from disk

    If end

If (Variable loadloop < Variable V58) then . . .

    Jump to subroutine "MakeLoad"

    Load wave W3 from disk

20   If end

If (Variable loadloop < Variable V58) then . . .

    Jump to subroutine "MakeLoad"

    Load wave W4 from disk

    If end

25 If (Variable loadloop < Variable V58) then . . .

    Jump to subroutine "MakeLoad"

    Load wave W5 from disk

    If end

If (Variable loadloop < Variable V58) then . . .

30   Jump to subroutine "MakeLoad"

    Load wave W6 from disk

    If end

If (Variable loadloop < Variable V58) then . . .

    Jump to subroutine "MakeLoad"

35   Load wave W7 from disk

    If end

Task:loadwave   Allow mouse activity

Task Begin

Choose Load Data_ AD1 Under Wave

Task:_Startup   Allow mouse activity
Task Begin
5   Variable tracenum = 0.00000
Insert "Valve Assignments

0   Ringer
1   5 uM GABA
2   10 uM GABA
10   3   50 uM

Task:printjrn   Allow mouse activity
Task Begin
Choose Print_ Journal1 Under Journal

15   ---

Task:clearjrn   Allow mouse activity
Task Begin
Choose Clear Journal1 Under Journal

---

20   Task:sethresh   Allow mouse activity
Task Begin
Open instruction number 3 in task analyze

Task:viewwave   Allow mouse activity
25   Task Begin
Choose Edit Values . . . Fip Under Wave

Task:getVnum   Allow mouse activity
Task Begin
30   String sVdrug = " " appended onto end of string " "
If (Control V0 == 1.000) then . . .
       String sVdrug = "Valve 0" appended onto end of string "String sVdrug"
       If end

If (Control V1 == 1.000) then . . .

    String sVdrug = "Valve 1" appended onto end of string "String sVdrug"

    If end

If (Control V2 == 1.000) then . . .

5   String sVdrug = "Valve 2" appended onto end of string "String sVdrug"

    If end

If (Control V3 == 1.000) then . . .

    String sVdrug = "Valve 3" appended onto end of string "String sVdrug"

    If end

10   If (Control V4 == 1.000) then . . .

    String sVdrug = "Valve 4" appended onto end of string "String sVdrug"

    If end

If (Control V5 == 1.000) then . . .

    String sVdrug = "Valve 5" appended onto end of string "String sVdrug"

15   If end

If (Control V6 == 1.000) then . . .

    String sVdrug = "Valve 6" appended onto end of string "String sVdrug"

    If end

If (Control V7 == 1.000) then . . .

20   String sVdrug = "Valve 7" appended onto end of string "String sVdrug"

    If end

If (Control V8 == 1.000) then . . .

    String sVdrug = "Valve 8" appended onto end of string "String sVdrug"

    If end

25   If (Control V9 == 1.000) then . . .

    String sVdrug = "Valve 9" appended onto end of string "String sVdrug"

    If end

If (Control V10 == 1.000) then . . .

    String sVdrug = "Valve 10" appended onto end of string "String sVdrug"

30   If end

If (Control V11 == 1.000) then . . .

    String sVdrug = "Valve 11" appended onto end of string "String sVdrug"

    If end

If (Control V12 == 1.000) then . . .

35   String sVdrug = "Valve 12" appended onto end of string "String sVdrug"

    If end

If (Control V13 == 1.000) then . . .

    String sVdrug = "Valve 13" appended onto end of string "String sVdrug"

    If end

If (Control V14 == 1.000) then . . .

        String sVdrug = "Valve 14" appended onto end of string "String sVdrug"

        If end

If (Control V15 == 1.000) then . . .

5   String sVdrug = "Valve 15" appended onto end of string "String sVdrug"

        If end

Task:smooth   Allow mouse activity

Task Begin

10 AD1 = Smooth (AD1, 3.00000)

Variable vDel Seg = Variable Marker4 - 0.07000

AD1 = Delete (AD1, variable vDelSeg, variable Marker4)

Task:setauto   Allow mouse activity

15 Task Begin

If (Control dEpiNum > 1.000) then . . .

        Control sAuto = 0.00000

        If end

---

20 Task:protocol   Allow mouse activity

Task Begin

Control bDR = 0.00000

Control bDMD = 0.00000

Control dEpiNum = 1.000

25 Control cVcom = 0.00000

Control cVStep = 0.00000

Control dWashVlv = 0.00000

Control cWashtim = 0.5000

Control sGain = 0.00000

30 Control bVpulse = 0.00000

If (Control bP30s == 1.000) then . . .

        Move marker M4 to the 30.0000 position

        Move marker M3 to the 20.0000 position

        Move marker M2 to the 10.0000 position

35   If end

If (Control bP60s == 1.000) then . . .

        Move marker M4 to the 60.0000 position

Move marker M3 to the 40.0000 position

Move marker M2 to the 20.0000 position

If end

If (Control bIntPerf == 1.000) then . . .

5

Move marker M4 to the 90.0000 position

Move marker M3 to the 60.0000 position

Move marker M2 to the 30.0000 position

Control cVcom = 0.00000

If end

10

Task:header   Allow mouse activity

Task Begin

If (Control bDR == 1.000) then . . .

Insert "----Start Dose Response----" into journal Journal1

15   Insert "

" into journal Journal1

Insert "

" into journal Journal1

If end

20   If (Control bDMD == 1.000) then . . .

Insert "----Start D/M/D Protocol----" into journal Journal1

Insert "

" into journal Journal1

Insert "

25   " into journal Journal1

If end

Task:flush   Allow mouse activity

Task Begin

30   Variable cnt4 = 1.000

While (Variable cnt4 <= control dEpi Num) do . . .

Variable flushlp = Control dAltvalv +Control dEpiNum

Variable flushlp = Variable flushlp - Variable cnt4

Variable Vflush = 1.000

35   Variable cntl = 0.00000

While (Variable cnt1 < Variable flushlp) do . . .

Variable Vflush = Variable Vflush * 2.000

Variable cnt1 = Variable cnt1 + 1.000

While end

Variable FlushOut = Variable Vflush + 1.000

Variable FlushOut = Variable FlushOut * -1.00

Cwrite (devType147, offset=192, slot=4; FlushOut to Value)

5   Delay for 3.00000 seconds

Cwrite (devType147, offset=192, slot=4; -1.00 to Value)

Variable cnt4 = Variable cnt4 + 1.000

While end

---

10   Task:oocytnew   Allow mouse activity

Task Begin

Insert "New oocyte #

Rp = -   at 1 min

" into journal Journal1

---

15   ------

Task:footer   Allow mouse activity

Task Begin

If (Control bDR == 1.000) then . . .

     Insert "----End Dose Response----" into journal Journal1

20   Insert "

" into journal Journal1

     Insert "

" into journal Journal1

     If end

25   If (Control bDMD == 1.000) then . . .

     Insert "----End D/M/D Protocol----" into journal Journal1

     Insert "

" into journal Journal1

     Insert "

30   " into journal Journal1

     If end

Task:showhead   Allow mouse activity

Task Begin

35   Choose Show jHeader Under Edit

Task:print_Jhd   Allow mouse activity

Task Begin

Choose Print_ jHeader Under Journal

5

Task:clearwav   Allow mouse activity

Task Begin

Set wave W1 internals (0.00000 to #valid data pts)

Set wave W2 internals (0.00000 to #valid data pts)

10   Set wave W3 internals (0.00000 to #valid data pts)

Set wave W4 internals (0.00000 to #valid data pts)

Set wave W5 internals (0.00000 to #valid data pts)

Set wave W6 internals (0.00000 to #valid data pts)

Set wave W7 internals (0.00000 to #valid data pts)

15

Task:MakeLoad   Allow mouse activity

Task Begin

Variable V57 = Variable V59 - Variable loadloop

String _LoadWav = "" appended onto end of string ""

20   String _LoadWav = date (e.g. "1/30/64")

String _LoadWav = ".AD1." appended onto end of string "String _LoadWav"

If (Variable V57 < 10.00) then . . .

    String _LoadWav = "0" appended onto end of string "String _LoadWav"

    If end

25   If (Variable V57 < 100.0) then . . .

    String _LoadWav = "0" appended onto end of string "String _LoadWav"

    If end

String _LoadWav = "Variable V57" appended onto end of string "String _LoadWav"

Variable loadloop = Variable loadloop + 1.000

30

Task:markjrnl   Allow mouse activity

Task Begin

Insert "-----------------------------

" into journal Journal1

35

Task:pRecord   Allow mouse activity

Task Begin

Move marker M4 to the 118.000 position

Move marker M3 to the 110.000 position

Move marker M2 to the 10.0000 position

5   Control cWashtim = 0.00000

Control dEpiNum = 2.000

Control cVcom = 0.00000

Task:pCL.1   Allow mouse activity

10   Task Begin

Move marker M4 to the 6.00000 position

Move marker M3 to the 4.00000 position

Move marker M2 to the 2.00000 position

Control cWashtim = 0.00000

15   Control dEpiNum = 10.00

Control cVcom = -50.0

Control cVStep = -10.0

Control sGain = 1.000

Control cWashtim = 0.00000

20   Control bVpulse = 1.000

Show Alert: "I(Cl) = V-dep. chloride current, activates on hyperpolarization

----

Task:pCL.2   Allow mouse activity

Task Begin

25   Move marker M4 to the 6.00000 position

Move marker M3 to the 4.00000 position

Move marker M2 to the 2.00000 position

Control cWashtim = 0.00000

Control dEpiNum = 4.000

30   Control cVcom = 100.0

Control cVStep = 10.00

Control sGain = 0.00000

Control cWashtim = 0.00000

Control bVpulse = 1.000

35   Show Alert: "I(Cl(Ca)) = calcium-dep. chloride current, activates on depolar

----

Task:pIVcurve   Allow mouse activity

Task Begin

Move marker M4 to the 6.00000 position

Move marker M3 to the 4.00000 position

Move marker M2 to the 2.00000 position

5   Control dEpiNum = 12.00

Control cVcom = 60.00

Control cVStep = -10.0

Control bGain = 0.00000

Control cWashtim = 0.00000

10   Control bMarkers = 1.000

Show Alert: "IV curve characterizes passive membrane (+10 mV to -100 mV). S

Task:pMemRes   Allow mouse activity

Task Begin

15   Move marker M4 to the 6.00000 position

Move marker M3 to the 4.00000 position

Move marker M2 to the 2.00000 position

Control dEpiNum = 1.000

Control cVCcom = 10.00

20   Control cVCstep = 0.00000

Control bGain = 0.00000

Control cWashtim = 0.00000

Control bMarkers = 1.000

Show Alert: "Membrane resistance measurement. Applies +10 mV pulse, set Vh=